

COLLEGE OF AGRICULTURE
AGRICULTURAL EXPERIMENT STATION
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BOVINE INFECTIOUS ABORTION
AND ASSOCIATED DISEASES OF
CATTLE AND NEW-BORN CALVES

I. METHODS OF CONTROLLING ABORTION
GEORGE H. HART

II. THE IMPORTANCE OF *BACTERIUM ABORTUM* OF BANG AND OTHER MICRO-
ORGANISMS IN BOVINE INFECTIOUS ABORTION
JACOB TRAUM

III. MANIFESTATIONS OF ABORTION DISEASE WITHOUT DEMONSTRABLE
ETIOLOGICAL FACTOR
JACOB TRAUM and GEORGE H. HART

IV. DISEASES OF THE GENITAL TRACT OF CATTLE FREQUENTLY ASSOCIATED
WITH ABORTION
FRED M. HAYES

V. THE DIAGNOSIS OF PREGNANCY IN CATTLE
GEORGE H. HART

VI. SCOURS AND PNEUMONIA IN CALVES
GEORGE H. HART and JACOB TRAUM

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CONTENTS

	PAGE
I. Methods of Controlling Abortion:.....	271
The Location of <i>Bacterium abortum</i> Organisms in the Body of Infected Animals.....	271
Escape and Life of the Organisms outside of the Body of Animals.....	274
Method of Drawing Blood for the Agglutination and Complement Fixation Tests.....	277
Discussion of this Method of Diagnosis.....	279
Procedure in Uninfected Herds.....	280
Procedure in Infected Herds.....	282
Outline of Vaccination Experiments Now Being Conducted by this Station.....	288
Abortion, a Self-Limiting Disease.....	290
Summary and Conclusions.....	291
II. The Importance of <i>Bacterium abortum</i> of Bang and Other Microorganisms in Bovine Infectious Abortion	294
Introduction.....	294
Review of the Investigations bearing on the Cause of Infectious Abortion of Cattle.....	296
Pathogenicity Tests of Various Strains of <i>Bacterium abortum</i>	308
Observations upon Forty Aborted Fetuses Received at Berkeley.....	309
Discussion.....	320
The Power of <i>Bacterium abortum</i> to Produce Manifestations of Abortion Disease.....	321
The Failures of <i>Bacterium abortum</i> to Produce Manifestations of Abortion Disease.....	321
The Extent of <i>Bacterium abortum</i> Infection.....	322
Other Organisms Connected with Manifestations of Abortion Disease.....	323
Conclusions.....	327
III. Manifestations of Abortion Disease Without Demonstrable Etiological Factor	
History of the Herd.....	327
Cases of Abortion.....	327
Summary of the Cases.....	330
Other Cases Showing Difficulties of Breeding.....	340
Feeding of the Herd.....	341
Condition of Animals to Date.....	342
Other Factors Associated with Breeding Difficulties as Possible Contributing Causes of Abortion.....	342
Febrile Diseases and Injury.....	342
Poisonous Plants, Spoiled Feed, and Alfalfa	343
Impaired Breeding Ability on the Part of the Bull.....	345
The Effect of Diet on Reproduction	345

	PAGES
IV. Associated Diseases of the Genital Tract of Cattle.....	347
Retained Placenta.....	348
Metritis and Endometritis.....	354
Pyometra.....	356
Cervicitis.....	358
Salpingitis.....	359
Cystic Degeneration of the Ovaries.....	362
Discussion.....	366
V. The Diagnosis of Pregnancy in Cattle.....	367
The Estrus Cycle.....	367
Changes taking Place in the Genital Tract during the Estrus Cycle	
Phases.....	368
Irregularities in the Estrus Cycle.....	370
Changes in the Genital Organs occurring in Pregnancy.....	373
External Manifestations of Pregnancy.....	377
Economic Value of the Knowledge of the Presence and Stage of Preg-	
nancy.....	377
Summary and Conclusions.....	379
VI. Scours and Pneumonia in Calves.....	380
Introduction.....	380
Bacterial Causes.....	381
Avenues of Infection.....	384
Incidence of the Disease.....	387
Factors aside from Bacterial Agents in the Causation of the Disease....	388
Prevention.....	390
Treatment.....	391
VII. References.....	393
VIII. Glossary.....	397

ILLUSTRATIONS

	PAGE
Figure 1. Non-pregnant Genital Tract of the Cow.....	293
Figure 2. Illustrating Method Used in Growing <i>Bacterium abortum</i> under CO ₂	310
Figure 3. Gravid Uterus of Cow at Full Term, Laid Open to Show Fetal Sac	
with Faint Outline of Fetus.....	326
Figure 4. A Cotyledon from a Pregnant Uterus.....	349
Figure 5. Enlarged Cystic Ovary on Left, with Normal Ovary on Right.....	363
Figure 6. Cross-Section of the Cystic Ovary (left) shown in Figure V.....	363
Figure 7. Cow with Long-standing Cystic Ovaries.....	364
Figure 8. Cow with Cystic Ovaries of Two Years' Standing.....	365
Figure 9. Microscopic Section of Ovary of Cow, Showing a Graafian Follicle	
with Ovum.....	369
Figure 10. Three Pairs of Ovaries from Cows, each Pair Showing an Ovary	
Containing a Corpus Luteum.....	371
Figure 11. Same as Figure 10, with the Ovaries Containing the Corpus Lutei	
Cut in Cross-Section.....	371
Figure 12. Microphotograph of Spermatozoa from a Bull, magnified x800.....	375

I

METHODS OF CONTROLLING ABORTION

BY GEORGE H. HART

THE LOCATION OF *BACTERIUM ABORTUM* ORGANISMS IN THE BODY OF INFECTED ANIMALS

The location in the animal body of the causative organism of abortion has been a very important fundamental problem in outlining methods for the control of the disease and one which has been the subject of much study and experimentation. W. L. Williams⁸⁹ in 1916, and again in 1921⁹⁰ carefully outlined the theory that calves in infected herds or calves fed raw milk containing *Bacterium abortum* or other organisms became infected, continued to harbor the organisms until they reached maturity, and were very liable to abort with their first pregnancies. This theory has been rather definitely refuted by experiments of Dick and Duebler¹⁸ in Pennsylvania, by Simms and Miller⁷¹ in Oregon, and by observations in various parts of this and other countries. Prior to and coincident with the refutation of this theory, experimental work designed to ascertain the sites in the body of the infected animal which constituted the seat of temporary and permanent location of the abortion organisms was carried on extensively. The result of this work has greatly increased our knowledge regarding this important phase of the disease. Today the work of Schroeder and Cotton^{65, 66, 67}, Mohler and Traum⁴⁹, Buck, Creech and Ladson⁸ in the U. S. Bureau of Animal Industry, and of other investigators has established the fact there are four definite locations of *Bacterium abortum* in the body of infected animals. It is important for owners of live stock to know something of these seats of localization and to understand how the organisms leave the body of one animal and gain access to the bodies of other animals.

Udder and Supramammary Lymph Glands.—The udder and the supramammary lymph glands are probably of first importance in this regard because in mature cows in which the udder has been active they become the more or less permanent seats of the organisms in a considerable percentage of infected animals. The organisms will remain in these glands and be given off very regularly in the milk for a period of years—in one cow, under the observation of

Schroeder⁶³, for a period of seven years. On the other hand, except in one case of apparently natural infection, reported by Carpenter¹⁰, and one case in an adult virgin four-year-old animal following intravenous inoculation by Schroeder⁶⁵, the organism has not been found in the undeveloped udder or supramammary lymph glands of unbred heifers. The general opinion supported by field results, is that in such animals, these glands are very rarely seats of the infection even though the animals may be exposed to it during the early period of their lives. Infection is at most so unusual as to be of little practical importance in formulating measures for the control of the disease.

Pregnant Uterus and Discharges From Genital Tract.—The second important place in which the organism is found is in the pregnant uterus and in discharges from the genital tract following abortion or normal parturition. The premature expulsion of the fetus is the final act in the chain of events following infection with the *Bacterium abortum* Bang. The organism may produce this event at any stage of gestation, from shortly after conception to the normal time for the birth of the calf. A certain period of time, which varies in individual animals, is required from the entrance of the organisms into the animal's body until abortion occurs, and the end of gestation may intervene before this has taken place. The offspring in such a case will be born apparently normal and alive, although the uterus, membranes, and discharges are teeming with the organisms. For this reason all animals in infected herds must at this time be looked upon as possible spreaders of the organism even though they have not and may never abort. According to Theobald Smith⁷³, who has made a very careful and enlightening histo-pathological study of the mode of entrance and changes produced by *Bacterium abortum* in the pregnant uterus of cattle, the bacteria are deposited in the maternal cotyledons by the blood stream. From here they pass through to the fetal cotyledons and gradually spread out over the epithelium of the chorion or external fetal membrane, and in this process they destroy the cells and cause them to be exfoliated. They then pass through into the amniotic fluid, and, in the physiological process of the swallowing of this fluid by the fetus, they are carried into the stomach, or they may reach the interior of the body of the fetus direct from the placenta through the umbilical vein. The cotyledons and membranes, therefore, become infected first in the progress of the bacteria and may be the only parts containing the organisms when gestation ends and parturition occurs. Experiments by Schroeder and Cotton⁶⁵ have rather definitely shown, on the other hand, that the non-pregnant uterus does not harbor the organisms

and that they leave an infected womb in a few weeks after parturition or abortion. The longest time it was found by them to remain in the womb was fifty-one days following the emptying of the uterus. Organisms in great numbers experimentally placed in the empty uterus remained there only a few days. Bulls experimentally infected by placing the organisms in the sheath and smearing them over the penis just prior to copulation did not transmit infection to the female. Thus it would appear that the empty uterus is not a favorable seat for the multiplication of the organisms even though they do gain access to it. This condition changes, however, after pregnancy, and McFadyean and Stockman⁴² and others have shown that experimentally cattle may become infected by the vaginal route after pregnancy has been established for some weeks or months.

Genital Tract of Bulls.—The third definite location of the organisms is in the genital tract or the accessory glands of a certain percentage of bulls. Here it may set up small foci of inflammation and abscess formation from which the organisms may constantly escape from the animal through the discharges from the genital tract. Even though the experiments of Schroeder and Cotton⁶⁷ tend to show that organisms excreted from such an animal into the vagina of the female at the time of copulation would not infect her, the animal would nevertheless be passing the organisms on to litter and other material which came in contact with his discharges. The lesions of the genital tract of the bull may be recognized on physical examination in certain rare cases, but failure to find such a condition is not definite evidence that the animal is free from infection. This condition may be determined by the agglutination test applied to the animal's blood.

Gastro-Intestinal Tract of Calves.—The fourth seat of the organism is the gastro-intestinal tract of calves during the periods when they are fed on infected milk. It is not generally believed that the organisms remain permanently in the bodies of these animals after they are weaned, because of the fact that the udder, the permanent seat of infection, is not subject to such infection until it becomes active prior to and following the first parturition. Definite experimental evidence that such animals excrete abortion bacilli with the intestinal discharges has not been established. As they are frequently ingesting the organisms in large numbers with the milk, however, this should be considered a possible means of spreading the infection until it is definitely proved otherwise.

Outside of these four locations, the organism is not found with any regularity in other parts of the animal body. Schroeder and

Cotton⁶⁵ have injected them in suspensions into the circulating blood and could not recover them from the blood after a period of two hours. In unborn infected fetuses and abortions, it is found with great regularity in the lungs and stomach contents, having reached the inside of the body through the blood stream from the cotyledons or in the physiological process of swallowing the amniotic fluid which becomes infected with the organisms after they have passed through the cotyledons and chorion.

ESCAPE AND LIFE OF THE ORGANISMS OUTSIDE OF THE BODIES OF ANIMALS

Since abortion is a chronic disease, its manifestations are not recognized for weeks or months after infection occurs, a fact that in some cases makes it difficult to trace the source of the infection. Not only is a knowledge of the location of the organisms in the animal body necessary, but also their method of escape and existence outside of the body are factors of equal importance in controlling the disease. Present knowledge leads us to believe that the organism is strictly parasitic and cannot multiply outside the body of a susceptible animal. It is found in nature only as the result of contamination with discharges from infected animals in which it is present. Under favorable conditions of moisture and protection from sunlight, however, it will live for a period of weeks or even months outside of the body of the host animal. An abortion occurring in pasture, it can readily be seen, offers an ideal means of spreading the organisms over the feed supply of the remainder of the herd. The same condition arises when abortion occurs around farm buildings or when the animal is allowed to mix indiscriminately with the herd in pasture or corral for several weeks following an abortion, while she may be still discharging the organisms from her vulva. Under such conditions animals have been seen to smell and actually lick up visible amounts of vaginal discharge from the ground. The throwing of infected litter and bedding from the stable into a corral is also a source of infection because of the very common habit of cattle to eat straw and other bedding soiled with urine and feces, from the ground of a corral even when good hay is available in feed racks. As the after-birth and uterine discharge may be teeming with the organisms even when parturition is normal, this is the period of time when all animals in infected herds must be looked upon as possible spreaders of the infection.

Bulls gaining access to the premises from outside sources may spread the organisms with discharges from their urethra. Although from our present knowledge we believe only a small percentage of bulls are so infected, their discharges are definitely known to be a source of such infection and therefore should be guarded against.

Calves during the milk drinking period may be passing the organisms with their feces and thus contaminating their surroundings.

Bacterium abortum is very frequently present in large numbers in milk, and care should therefore be exercised not to allow such milk to become a source of infection. Possible opportunity for such a means of infecting herds to occur under ordinary farm conditions is present when unpasteurized skim-milk from creameries is taken to uninfected premises; also when excess skim-milk and overflow milk from a creamery or skimming station are allowed to run into a drain pipe which opens into a flowing stream. Organisms from such a stream passing through pasture lands and furnishing drinking water may be taken into the bodies of susceptible animals even at a distance of several miles from the plant. It is possible for infection to be carried from a cow giving off the organisms with her milk, to a susceptible cow, on the hands of the milker or the teat cups of a milking machine, in which event the infection would enter the uninfected animal by way of the teat duct and establish itself first in the udder.

It should therefore be recognized that there are many ways by which the organism, after leaving the body of an infected animal, by one of the comparatively few and well understood channels, may gain access to the surroundings of other even remotely situated animals and set up a new focus of the disease.

The direct route, however, by which animals become infected with the organism under practical ranch conditions in the vast majority of cases is by way of the digestive tract through the ingestion of contaminated food. The original contamination on the uninfected ranch occurs through the purchase and addition to the clean herd of one or more animals infected with the organisms and giving them off from their bodies without necessarily having themselves any observable breeding difficulties. Experimental evidence shows that animals may become infected in other ways, such as by way of the vagina after pregnancy has become established; by way of the teat duct and udder, (Schroeder and Cotton⁶⁵), by way of the conjunctiva (Seddon⁶⁸), in laboratory animals; but these sources of infection probably do not exist or are of only secondary importance under ordinary conditions of handling cattle on the farm.

The direct transmission of the infection from the male to the female, or vice versa in the act of copulation is no longer held to be an important means of spreading the disease, if it is a factor at all. This addition to our knowledge regarding the disease minimizes the importance of the one-time widespread recommendation and frequent application of the routine practice of douching the sheath of the bull before and after each service. Such treatment at best only disinfects the sheath and exterior of the penis, while in infected bulls the organisms have been found in the seminal vesicles and epididymis where they cannot be reached by disinfectants and from which they are discharged into the vagina with the semen at the time of copulation.

The important factor to recognize, therefore, is that the infection is spread from herd to herd largely by the addition to the uninfected herd of infected animals from outside sources. The great amount of movement of live stock in the ordinary channels of trade has been the means of spreading this disease, as well as the other great chronic scourge, tuberculosis, among the live stock throughout the whole state and country. Frequently an unscrupulous owner of live stock, despite a knowledge of the existence of the disease in his herd, has advertised it for sale and through the medium of the public auction has distributed the animals in small groups or individually to many new premises, so that a high percentage of the dairy herds of any size, and many of the range herds, have become infected with the disease. Live stock men must recognize the importance of this means of spreading disease and help to develop proper regulations to prevent it. Owners have too often in the past looked upon regulations for disease control as unnecessary hindrances to the operation of their business. The success of the Federal Accredited Herd Plan in tuberculosis eradication is evidence that coöperation of cattle men in solving disease problems leads to success and the consequent financial stabilizing of the industry. When purchases are to be made—and it is fully recognized that this is and always will be very frequently necessary for the continuation of the live stock business—certain efforts should be made to see that the animals have a good breeding history and that they are free from infection. The agglutination and the complement fixation tests are means at our disposal of helping to ascertain whether or not infection exists in animals to be purchased.

METHOD OF DRAWING BLOOD FOR THE AGGLUTINATION AND
COMPLEMENT FIXATION TESTS

Drawing blood is an operation that is being more and more commonly carried out and at times must be done in the absence of veterinary assistance. The apparatus required depends on the point from which the blood is to be taken. It may be collected from the ear, the tail, or the jugular vein. From dairy cows confined in stanchions, the jugular vein offers the most convenient source from which to draw it, by means of a sharp hypodermic needle such as is used in a blackleg or hog cholera syringe. These needles should be boiled before use. In going from cow to cow, however, it is only necessary to wash them out with disinfectant solution, followed by clear tap water. Care must be exercised that the lumen is open before inserting them, as otherwise no blood will flow when the vein is tapped. Wire stilets for cleaning particles of blood clots from the lumen should be at hand. The most desirable needle for the purpose is one with the stem about two inches long and from fourteen to sixteen gauge.

The animal's head is restrained by an assistant with the fingers of one hand in the nostrils, or with a halter, or nose tongs, pulling the nose around to the right side and, if necessary, tying it to the upper cross-piece supporting the stanchion. If long hair is present on the left side of the neck, clip it with scissors or clippers and sponge the area with 2 per cent compound solution of cresol or other similar disinfectant. With the thumb of the left hand press upon the left jugular groove, which will cause the jugular vein to enlarge by retarding the flow of blood to the heart so that it can be distinctly seen. With the needle held firmly in the right hand, it is inserted directly over the center of the swollen vein. A strong push is required to insert even a sharp needle through the thick skin of cattle as compared to the much thinner hide of the horse. It is rare for the needle to be successfully pushed through the skin and into the vein at the same time. In case it has been inserted directly over the center and in the same general direction of the vein, a second sharp thrust, with the left hand keeping the vein well distended, will usually carry it in. Blood will then flow freely from the needle and should be collected in clean dry glass vials or bottles. It is very important that the containers be dry as a very small amount of moisture will cause the blood to hemolyze, thereby coloring the serum, which may form a precipitate or otherwise render interpretation of the test

difficult or impossible. About 15 mils (one tablespoon) of blood is a satisfactory amount; when difficulty is experienced in getting it to flow readily, a less amount can be made to suffice, but in no case should this be less than one teaspoonful.

A list for identification of the cows may be kept on paper and the bottles simply numbered on the corks, or, if a label is available, the cow's description and number may be placed on it and the label pasted on the bottle.

After the blood has clotted in the bottles, they should be shaken or sharply struck against the palm of the hand to loosen the clot from the sides, allowing it to contract and the serum to separate.

In animals which have to be handled in chutes—thick-necked bulls and the beef breeds—it is difficult to make the jugular vein stand out with sufficient prominence and also difficult for the operator to be in a position to get the needle into it. Under these conditions blood must be drawn from the ear or tail. In valuable animals where it is not desirable to slit the cartilage of the ear, the hair may be clipped short on the upper surface of the ear and a vein cut without cutting through the cartilage. The principal objections to ear bleeding is the small amount of blood which usually flows from the incision and the time required to get a number of samples.

The tail therefore offers a more favorable source under these conditions. In drawing blood from this appendage, the incision is made on the under surface, as the blood vessels of the tail pass along the under surface and to each side of the coccygeal vertebrae. The incision should be made with a sharp knife and preferably over the center of the vertebrae, rather than at the end, so as to avoid the possibility of opening a joint. Any point on the tail above the brush may be selected as the point of incision. When the artery is completely severed, blood flows very freely and, after sufficient is collected the hand should be held over the incision to stop excessive bleeding. Sometimes it is necessary to wrap the incision with cotton and a bandage for a day or two. With experience, the incision can be made deep enough in most cases to get sufficient blood without producing an excessive flow. There is no danger of an animal bleeding to death from such an incision; the wound in all cases under our observation has healed readily, leaving practically no scar.

The blood samples should be sent by express at once to the laboratory. In warm weather or when long shipments are necessary, it is preferable to let the clot contract over night in a cool place and then decant the clear serum off into clean dry bottles and ship. Only the clear serum is used in making the test; when this cannot be obtained,

the reading of the test is interfered with. When decanting cannot be carried out, the whole blood, during shipment in warm weather should be iced.

DISCUSSION OF THIS METHOD OF DIAGNOSIS

While the agglutination and complement fixation tests have a definite place in the control of abortion and will undoubtedly come into much wider use in the future than they have in the past, live stock owners should realize the limitations of the procedure. The agglutination test, which is the one usually employed, consists in bringing small amounts of the serum of the animal to be tested in contact with a suspension of abortion organisms in physiological salt solution in test tubes and allowing it to stand for twenty-four to forty-eight hours. In case the serum shows a positive reaction, the blood of the animal will contain what are termed agglutinins, and these will agglutinate the abortion organisms so that they will clump together in the bottom of the tube, leaving the supernatant physiological salt solution clear. If the cow is not a reactor, her serum will contain no agglutinins, the abortion organisms will not become clumped, and the fluid will remain cloudy, the condition it is in when the serum is added. The agglutinins are specific for the infecting organisms and will agglutinate no others. A positive reaction to the agglutination test indicates that the animal from which the blood was drawn is, or recently has been infected with the disease. It is no proof that she will abort, because some animals become infected with *Bacterium abortum* and never abort. These animals may, however, spread the disease to other animals, as already mentioned.

In testing blood from whole herds of animals, the agglutination test gives an idea of the extent of the infection in the herd. Animals giving a positive reaction are the ones most likely to abort in the future. According to Schroeder, about 60 per cent of the positive reactors will expel the organisms with their milk. Some animals shortly after abortion will give a negative agglutination test. In two first-calf cows under our control which had probably never aborted, a suspicious reaction to the agglutination test was obtained. They were sent to be slaughtered, and blood taken at time of killing was negative. Despite this finding, *Bacterium abortum* was present in the supramammary lymph glands of both animals. Single negative reactions are, therefore, not positive proof of absence of infection. Negative reactions of whole herds are much more valuable.

Isolation or removal of positive reactors, where they are so few in number as to make it practical, with repeated agglutination tests on the remainder, may successfully remove infection in certain selected cases. The test is rendered temporarily or permanently valueless by the previous administration of killed abortion organisms, in the form of bacterins, or of live abortion organisms in the form of vaccine.

PROCEDURE IN UNINFECTED HERDS

The recommendations to owners of live stock in regard to this disease vary more or less in each individual case. In dealing with a disease of such widespread nature, the conditions met with vary exceedingly. Thus, in one part of the state we may see a herd of several hundred head of range cattle to which no additions are made except bulls from time to time. These animals are constantly kept on patented land and do not mix with other animals. The range may be divided into several isolated pastures or be practically all in one. Owners of other similar herds take their cattle to the Forest Reserve during the summer season, at which time they are in more or less intimate association with other cattle. These are very different situations from that confronting the owner of a dairy herd kept constantly corralled on a comparatively small piece of high-priced land with no opportunity to segregate groups of animals or to raise young stock. Pure-bred herds offer problems entirely different from grade herds. It is therefore very difficult to cover these extremes and the gradations between them in such a way that the recommended procedures will not appear impractical to some owners. The fact remains, however, that the condition desired in all cases depends on the principles deduced from the careful study and experimentation of many investigators in various parts of the world and on the results gained from field experience in applying these principles in the actual handling of herds. In many cases the ideal procedure cannot be carried out, but, if the ideal is recognized, it can be approximated as nearly as the individual circumstances render possible, and satisfactory, partial, or poor results expected accordingly.

The disease is very widespread—statements have been made by some that practically no large dairy herds are free from it. But when we consider that if new infection is excluded, the disease may run its course in infected herds, then it is perfectly proper for an owner to assume that his herd is no longer infected and to proceed accordingly. As the greatest source of spreading the abortion infection is the adding of new stock from infected herds, the fundamental

recommendation is to raise sufficient heifers to replace the adult animals and thus reduce the purchase of animals to a minimum. When this cannot be done, the safest class of females to add are those between weaning age and breeding age. Animals during this period of their lives are least likely to carry the organism in their bodies. It is generally believed and has been proved in practical experience, that these animals so rarely carry infection that under all ordinary conditions this is a safe procedure. However, *Bacterium abortum* has been isolated in rare cases from the udder of heifers which have never lactated (Carpenter¹⁰ and Schroeder⁶⁵).

Agglutination tests recently reported by Barnes⁵ showed 13.4 per cent positive reactions in eighty-two unbred heifers over one year of age from seven herds, and 19 per cent of twenty-six between six months and one year of age from five herds. Therefore, where all precautions are being taken, such heifers should have their blood tested. This knowledge that females rarely carry the organisms permanently in their bodies until it has found lodgment in the udder and supramammary lymph glands—and this occurs only after the gland has been actively engaged in the secretion of milk—does not apply to the male. Our knowledge is at present less definite with regard to this sex. It has been conclusively demonstrated that the organisms may secure a permanent foothold and set up pathological processes in the male genital tract without in any observable way interfering with the general health or breeding ability of the animal. At just what age this may occur is not definitely known and while in those cases in which this condition has been reported the animals were sexually mature, it is known that bulls become sexually mature at an early age. Therefore, on adding bulls past the weaning age to clean herds, they should first have the agglutination test applied.

In both pure-bred and grade herds it is necessary from time to time to add adult females from outside sources. It is with such animals that infection is most liable to be introduced. While adult females from close-by herds, with whose breeding history the purchaser is acquainted, may be added with comparative safety, it is a good general rule of procedure to have the blood of such animals tested. Too much dependence cannot be placed on single negative tests in such animals, and when little is known of the breeding history and conditions render it possible, a second examination of their blood should be made after thirty to sixty days. This is recommended even though the animals cannot be kept isolated in the meantime.

Owners of clean herds are frequently faced with two other situations which may become sources of infection. One of these is sending

females away to be bred, and the other is admitting outside females to the premises for breeding to the herd sire. As already stated, there is a considerable amount of evidence at hand today to show that the act of copulation in itself is not a means of spreading infection from female to male, or vice versa. The danger is rather that outside cows will spread infection on the premises, either from vaginal discharge containing the organism, when the cows are brought to be bred too soon after abortion or parturition, or by contamination of the food of the home animals; by contact with milk of the outside cows. Females going away to infected ranches are more liable to become infected by ingesting food contaminated with the organism than by service from the bull. Cows brought on premises to be bred should therefore remain on neutral territory, i.e., territory not occupied by the home animals, and at least two months should have elapsed since parturition. Animals sent away to be bred in herds, the abortion history of which is unknown, should be sent only to places where proper precautions are taken against infection.

Finally, owners of uninfected herds should watch the water supply and drainage conditions as a possible source of contamination.

Under range conditions, where cattle are taken into Forest Reserve range and there mingle with other herds, the opportunities for keeping out infection are difficult. Even under such conditions, it is common for a group of a comparatively few cattlemen to largely control the range in particular valleys and meadows, and these men should consider as a group, the abortion problem, at least during the period of the year when their cattle are running together.

In small herds which are members of bull clubs, it is also necessary to consider all the animals in such a club as a unit in dealing with this disease.

PROCEDURE IN INFECTED HERDS

The degree by which the presence of infectious abortion in a herd manifests itself in actual abortion varies widely. In some cases it spreads through the herd until the incidence of premature expulsion of the fetus reaches 50 to 75 per cent of the pregnant animals; in a few rare instances, as high as 90 per cent have been known to abort. In other cases the disease seems to spread slowly, never becoming a serious factor in the calf crop or dairy production, and, in a few rare instances in small herds, a single case of abortion definitely due to *Bacterium abortum* Bang is all that has been observed to occur. Mild infections sometimes develop sudden virulence and cause great losses. This phenomenon has been termed by Williams "abortion

storms," and the potential danger of such an infection should not be underestimated.

On account of the fact that abortion is a self-limiting disease, many substances have received widespread credit as curative agents without having any practical value as such. Among these may be mentioned the carbolic acid and methylene blue treatments and there is a host of proprietary preparations. Any method outlined for controlling this infection in the herd must be carried out on a fairly large scale before definite conclusions can be deduced as to its merit and credit given for good results. Each infected herd offers problems peculiar to itself, and considerable thought should be given by the owner and his veterinarian to the method to be followed in limiting losses. They should familiarize themselves with an early diagnosis of abortion due to *Bacterium abortum* Bang, and all cases of abortion should be considered to be of this nature unless they are definitely proved otherwise. If a laboratory is close at hand, the fetus and membranes can be examined for the presence of the organisms. The more practical method of ascertaining definite knowledge of the presence of the infection consists in having the agglutination test applied to the blood of the suspected animals.

Three distinct methods of procedure for controlling the disease are outlined below, either one of which may be used alone in certain selected cases, or they may be combined to advantage when it appears that better results are to be obtained thereby. These methods are:

1. The repeated testing of the entire herd by the agglutination test, and the removal of reactors.
2. The isolation method.
3. The vaccination method.

In average-sized herds in which the infection appears, blood should be taken from all the animals of breeding age, male and female. In case this shows but a very small percentage of infected animals and other conditions are favorable, it is feasible to expect that good results will follow the use of the first method for controlling the disease.

Repeated testing of the entire herd by the Agglutination Test, and removal of reactors.—Actual details of handling abortion under this method are rare in published reports. The work of E. M. Robinson⁶¹ in South Africa is therefore of great interest in this regard. He experimentally demonstrated the feasibility of eradicating the infection from a herd by this method. The experimental herd, consisting of sixty-three cattle—cows, heifers, and one bull, taken from a herd of one hundred head—were taken to a clean farm. The remainder

of the herd consisted of aborters and reactors, except for one positive cow which, at the time they were moved in 1915, gave a positive agglutination test and was accidentally included. In January, 1916, a test was applied to all the animals and this cow and four others reacted positively. These animals were isolated, and two aborted, one was barren, and two calved normally but the presence of abortion organisms were demonstrated in their milk.

Another agglutination test in March, 1916, showed three more reactors. These were removed; one aborted, one calved normally, and one did not breed. After this test, the reactors were no longer isolated on the farm, but removed to different premises.

A third test of the remaining cattle was made in May, 1916, and showed only one positive reactor. This cow was removed and aborted in July of the same year.

In July, 1916, a fourth test was applied to the herd and showed two reactors. Both of these cows were removed. One calved normally and one gave birth to a premature living calf. *Bacterium abortum* was isolated from the milk of both animals.

In August, 1916, a fifth test was made and there were no positive reactors. Two years later there had still been no positive reactors in this herd or among the clean herd into which they were introduced.

This experiment shows that the animals reacting positively are the most likely ones to abort and rather frequently are giving off the organisms from their bodies.

By the application of five agglutination tests and the removal of eleven head from the herd of sixty-three animals, over a period of about eight months, the infection was eradicated from the herd.

An effort to apply this method would be practical for owners with two or more herds on different ranches, and for those with a small percentage of reactors where opportunity exists to raise young stock to replace the adult cows, thus rendering outside purchases unnecessary. With the more general application of the agglutination test to entire herds of cattle, opportunities for demonstrating the value of this plan will undoubtedly present themselves.

The Isolation Method.—It is under this plan that we believe the disease can be handled to the best advantage in the great majority of herds. It is based on our knowledge of the means of escape of the organisms from the bodies of infected animals and consists in so handling the animals that the escaping organisms will have least opportunity of gaining access to the bodies of uninfected animals. To do this, isolated maternity quarters must be provided in which animals about to abort or calve normally are kept. They should

preferably be provided with entirely separate stalls, opening to the outside only, for individual animals, and should be disinfected or fumigated after each animal is removed from them. Bedding, after-birth, and drainage from such quarters should be burned, buried, or so handled that cattle cannot gain access to them. Such animals should be kept isolated from the herd until all discharge from the genital tract has ceased and, preferably until an examination by a veterinarian shows the genital organs to be in a normal condition. This has the double advantage of keeping the animals from spreading infection and making possible the recognition of abnormal conditions, which may thus be treated at once. The earlier such conditions are properly treated, the less is the danger of the animal becoming permanently sterile. Even though it is practically possible to keep such animals isolated only during the comparatively short period when their milk is unfit for commercial use, it will be advantageous to do so.

Calves, during the milk-drinking period, should be kept isolated from the remainder of the herd, and manure from their quarters should be so handled that cattle cannot come in contact with it or their food and water become contaminated by it.

Another source of contamination is carelessly-handled milk. Milk house drainage, contaminated with spilled milk, opening into irrigation ditches, or on to green alfalfa pasture, or alfalfa cut and fed green is a possible source of infection to well animals. During the general application of this method, the buildings and surrounding premises should be disinfected weekly, monthly, or quarterly, depending on the amount of infection present and the degree of completeness of the isolation methods which practical conditions render possible of maintenance.

Unbred heifers past the weaning age should be kept isolated, and, particularly, bred heifers should be separated from adult cows during the period of pregnancy. If only two groups can be maintained, unbred and bred heifers should be kept together, and separated from the cows. The herd sire, if a non-reactor, may be used to breed such animals with comparative safety if they are taken to neutral clean ground for breeding. Under these conditions, bulls giving a positive agglutination test should be eliminated from the herd. If positively reacting high-priced bulls must be maintained for breeding the adult cows, they should be kept corralled and given the necessary exercise in the corral or in such a way that possibly infected discharges from their genital tract will not contaminate the feed of other animals in the herd. Too great stress cannot be laid on the importance of this method of handling abortion. It is the means of

obtaining successful results, as has been demonstrated by field trials in various parts of the world and particularly reported upon in this country by Simms and Miller⁷¹ in Oregon, and by Dick¹⁸ and Duebler¹⁷ in Pennsylvania.

The Vaccination Method.—Three bacteriological products have been prepared for use in herds infected with this disease. These are dead abortion germs known under the name of abortion bacterins, live abortion germs known as abortion vaccine, and abortion serum made from the blood of cattle or horses immunized against *Bacterium abortum* Bang. One other bacteriological preparation known as abortin should be mentioned here. This was first prepared by McFadyean and Stockman in England by a method very similar to the method of preparing tuberculin and mallein. It was further studied and its method of preparation refined in this country by Meyer and Hardenbergh⁴⁷ in 1913. At the present time no definite field of usefulness for this product in the diagnosis or handling of abortion disease can be recognized.

Abortion Bacterin.—The first of these products to be widely placed on the market was abortion bacterin. It contained a suspension in salt solution or other fluid of abortion organisms grown on cultures in the laboratory and killed by means of heat. This being the first bacteriological product put out for the disease, it was exploited by commercial laboratories and was very widely used. As was to be expected, losses in some herds stopped after it was administered, but this occurs in a certain percentage of outbreaks when no treatment is given. Carefully checked experiments to ascertain its value have been carried out in England and Germany, and by the Wisconsin and Michigan agricultural experiment stations in this country. The conclusion reached in all cases was that no value can be attributed to the administration of this product.

Abortion Vaccine.—Abortion vaccine, consisting of live abortion germs, was originally introduced and used experimentally in sheep, goats, and cattle by Bang⁴ who obtained definite protective results in his carefully controlled experiments. Later it was studied extensively and used in the field by McFadyean and Stockman of the British Ministry of Agriculture. It is at present being distributed by this governmental agency to owners of live stock in England. It is produced commercially and distributed by a number of firms manufacturing biological products in the United States.

Carefully checked experiments by Hadley²⁵ of Wisconsin, Huddleson³³ of Michigan, and the U. S. Bureau of Animal Industry, as reported by Schroeder⁶³, seem to definitely show that its adminis-

tration is followed by a lower incidence of abortion in the treated than in unvaccinated control animals. Thus, in Hadley's experiments of 127 unbred heifers vaccinated, 77.9 per cent calved normally while only 66.7 per cent of the controls did so. The best results were obtained in open cows which had never aborted. In this group the vaccine was 91.8 per cent effective, which was in marked contrast to 44.4 per cent of normal calvings for the controls. In open cows which had aborted, the vaccine was of little value, and, as was to be expected, in cows which were pregnant at the time of vaccination but had never aborted, there was a higher percentage of abortions in the vaccinated than in the controls. The total vaccinated cattle from which his data were obtained numbered 439 head, of which 14.1 per cent aborted, while in the 101 controls 31.2 per cent aborted. The results varied in different groups; the highly favorable ones were obtained in the group consisting of open adult cows which had never aborted. The vaccinated cattle also showed a lower sterility rate and an increased breeding efficiency over the controls. Despite the comparatively large number of animals in the experiment, the writer warns against the danger of passing premature judgment on the value of any therapeutic agent for contagious abortion.

Huddleson's experiments cover a much smaller number of animals and the animals were not under such close observation, but he concludes in regard to Herd A: "These data apparently indicate a decrease in the abortion and sterility rate of the treated animals and a marked increase in the breeding efficiency of the treated over the untreated animals." In regard to his work on both herds A and B he states: "These data while very suggestive are too few to warrant final conclusions as to the value of vaccine treatment."

Schroeder's report covers twenty-three cattle which were part of a drove of sixty-six, none of which, according to the tests that could be made, was infected with bovine infectious abortion. Eleven received subcutaneous injection of pure living cultures of *Bacterium abortum* about two months before they were served by the bull. Four received repeated injections of killed cultures of the abortion organism after they had become pregnant, and eight were retained untreated as controls. The twenty-three cattle were equally and similarly exposed to abortion infection. The exposure was via the digestive tract and the material used was obtained from actual cases of infectious abortion. Of the eleven cows treated with live organisms before conception, ten calved normally and one aborted; of the four that received dead organisms, two calved normally and two aborted; seven of the eight controls aborted.

Despite the general concensus of opinion of investigators that the vaccine does reduce the incidence of actual abortion, and despite its continued distribution by the British Government over a number of years, the situation with regard to each individual infected herd should be considered thoroughly before this procedure is resorted to. This method is diametrically opposite to that outlined in the first plan. While there are conditions in which it is to be recommended, they certainly do not include any of those herds which might offer an opportunity to try out the first plan. Neither is it to be recommended for herds from which animals are constantly being sold for dairy or breeding purposes to go into possibly uninfected herds.

In our opinion, owners are justified in using this method in heavily infected herds with a high rate of actual abortions, when the animals are confined in corrals on small pieces of land with no opportunity to segregate groups of animals or raise young stock. Additions to the herd from outside sources are therefore constantly necessary, and sales of animals from such premises are usually for immediate slaughter.

Vaccine treatment is the simplest method of handling the disease and therefore the one which owners and veterinarians are liable to use without full consideration of the ultimate results. It is at best a means of reducing the manifestations of the disease, but does not eliminate infection from the herd. Reported results from herds so handled do not give information as to the number of vaccinated animals which become permanent carriers of this infection, nor mention subsequent history over a period of years following the treatment, which is essential before this can be recommended, on a wholesale scale.

OUTLINE OF VACCINATION EXPERIMENTS NOW BEING CONDUCTED BY THIS STATION

In order to obtain more information in regard to this method of treatment, a portion of the special abortion investigation appropriation made at the last session of the legislature is being used in the following outlined experiments which are now under way.

This investigation is designed to furnish information on the important and still unsettled question of the actual value of live abortion organisms in producing immunity. It is expected that it will throw additional light on the localization of the injected bacteria and determine whether or not it is necessary, in the production of immunity in *Bacterium abortum* infection, to have actual multiplication and activity of the organism in the animal body, or whether it is an immunity

that is conferred upon an animal simply as the result of having been infected with cultures of the causative agent.

It is expected that this investigation will also show the extent to which the infection, resulting from both the inoculation to produce immunity and the ingestion to produce infection, may be injurious to the animals infected and also to animals associated with them.

The question as to whether transmission of infection by the bulls, from infected groups to non-infected check groups, is likely to occur will be of great interest.

There have been purchased fifty-five heifers of breeding age and two young bulls known to be free from abortion infection as determined by history and laboratory tests. These will be divided into four groups:

Group 1.—Twenty heifers to be inoculated subcutaneously with live *Bacterium abortum* organisms prepared after the method used in the laboratory of the British Ministry of Agriculture. After two months, these animals are to be bred by placing the two bulls in the pasture with them. These bulls will have already served animals in Group 2. After pregnancy has been established, they are to be subjected to infection by the ingestion of *Bacterium abortum* organisms. This is the principal experimental group to determine the efficiency of live abortion organisms in the prevention of abortion.

Group 2.—Fifteen animals to be bred without any preliminary treatment by the two bulls before they are placed in Group 1. Ten of these animals, after pregnancy has become established, are to be subjected to infection with *Bacterium abortum* organisms in the same manner as Group 1. Five are to be kept as association animals with Groups 1 and 2. This is to be a check on the ability of our infective methods to produce abortion and on the control of the experiment in Group 1; also, an experiment with the five head on the extent to which pregnant cattle are in danger of becoming infected when exposed to aborting cows.

Group 3.—Ten animals are to be vaccinated and bred at the same time and in the same manner as Group 1, but not to receive any further treatment until after parturition. This group is to be examined after parturition for the presence of *Bacterium abortum* in the milk as a result of the vaccination. Some or all of these animals may be slaughtered after the milk examination is completed and attempts made to isolate *Bacterium abortum* from the internal organs.

Group 4.—Ten animals, five of which are to be bred, without previous treatment, by the bulls after they have served the animals in Groups 1, 2 and 3. We intend to have the other five animals left open so that in case opportunity offers, the bulls can serve them shortly after having served an aborting cow. This group, therefore, is to be a check on the bulls which have served vaccinated or recently aborting cows acting as carriers to non-infected females through the medium of copulation.

As an aid to the field observations on these four groups, laboratory tests of the blood of the fifty-five animals will be made at frequent intervals.

The germs used for infecting the thirty animals are to be obtained from such sources as will constitute, as nearly as possible, the means of spreading the organism from the carrier of the infection, such as the milk of several cows infected with *Bacterium abortum* and aborted fetuses containing the organism. *Bacterium abortum* of known virulence from laboratory cultures will also be given.

During this experiment, bacteriological examination of aborted fetuses from various ranches will be made. It is the intention in this work to examine approximately one hundred fetuses in order to ascertain the frequency in this section of the country with which *Bacterium abortum* Bang or other organisms are associated with the premature expulsion of the young. In this way, it will be possible to keep in constant touch with a source of supply of infective material of various kinds.

Abortion Serum.—If this material has any immunizing value it is of a passive nature and will not last for a longer period than a few weeks. Recently a theoretical field of usefulness for it has been brought forward in combination with dead abortion germs for safely carrying animals in infected herds through the latter months of pregnancy. No checked evidence is at hand to show that it has any value in this connection. Its field of usefulness under our present knowledge is restricted to very narrow limits.

ABORTION A SELF-LIMITING DISEASE

Despite the excessive losses it causes to the live stock industry and the widespread distribution of this infection, the fact should always be kept in mind that from the standpoint of the individual herd it is a self-limiting disease. This is due to the gradual acquiring of a tolerance or immunity to the particular strain of the organism, and, provided no new infection is added from the outside, it will cease to produce its manifestations over a varying period of time even though no special precautions are taken to limit its ravages. The period of time required for this varies greatly and is apparently longer in dairy herds than in beef herds. The latter are the class of herds where isolation methods are the most difficult to carry out. In such cases advantage should be taken of the natural production of immunity, and it is believed that this may be hastened by selection and breeding in such a way that animals which breed normally in

spite of the presence of the infection and those which develop immunity after one abortion can be kept for breeding. Those that are seriously affected by the disease and have retained afterbirth or abort a second time, or are difficult to get with calf and so on, may be culled out. Observations made by Potter⁵⁷, in Kansas, on range cattle are particularly interesting in this regard.

Herd immunity has been observed by all students of this disease in the field and its development is the reason that so many worthless substances have acquired a reputation as curative agents. In a large number of herds it is prevented from manifesting itself by the constant bringing in of fresh infection with the purchase of outside animals.

In the certified dairies around the San Francisco Bay region there are at present about 1800 head of dairy cattle. In order to keep out tuberculosis, it has been the practice for the past seven or eight years to raise sufficient young stock in these dairies to replace the old cows, which has reduced the purchase of outside cattle to a minimum. This has automatically reduced losses from abortion to a point where it is of little financial significance.

Therefore the presence of the disease to a severe extent in any particular herd where practical conditions render it impossible to introduce the recognized control measures should not necessarily cause the owner to hastily consign all the animals to slaughter. In some instances where the disease appeared in very virulent and widespread form, it has been known to clear up to a remarkable extent over a period of a single year. In the progress of the disease in a herd a certain percentage of animals are left temporarily or permanently sterile, which greatly adds to the financial loss it causes.

SUMMARY AND CONCLUSIONS

The localization of *Bacterium abortum* Bang in the bodies of infected bovine animals and the means by which it leaves the body are fairly well understood.

A knowledge of these fundamental facts is essential on the part of live stock owners to prevent infection from getting into the herd and to control it after it has gained access.

The agglutination and complement fixation tests, although they have their limitations, are important factors in furnishing information in regard to the status of individual animals and particularly individual herds in regard to the presence or absence of infection and the amount of same.

The greatest source of infection with the disease and the means by which it is introduced into herds in the vast majority of cases is the addition of infected cattle. Other more remote means exist but they always involve the contamination of surroundings with discharges from infected cattle and the transportation of the contaminated material to a point where cattle gain access to it without in the meantime exposing it to conditions that will destroy the organisms. Food and water supply are the great vehicles for so transporting the infectious agent.

Animals purchased from outside sources and community pasture or ranges are always a potential danger of the introduction of the disease. Depending on the practical conditions involved, danger from this source may be more or less completely eliminated.

When infection gains access to the herd definite information is at hand on the means to be taken to limit the losses from its presence.

Isolation methods of dealing with the disease are first to be recommended.

While under certain conditions the use of live abortion germ vaccine can be recommended after due consideration of all the factors involved, this course of procedure should not be adopted by owners without full knowledge of the facts and possible consequences.

The use of dead abortion germs is very generally believed to be worthless.

Less is known regarding the possible value of abortion serum, but it is not probable that it will ever take an important place as a means of controlling or preventing this disease.

The disease in any herd is self-limiting provided new infection is not constantly added and in a high percentage of herds will in time be reduced to a minimum even though very meager precautions are taken against it. This important fact renders it unwise to quickly dispose of whole herds of cattle because the infection is present in them.

In range cattle the self-limiting aspect of the disease seems to be more noticeable or manifests itself more quickly than in high-producing, closely-housed dairy herds.

Bulls containing *Bacterium abortum* in their discharges, while they may not infect cows during the act of copulation, are nevertheless spreaders of the bacteria contained in their discharges.



Fig. 1.—Non-pregnant genital tract of the cow.

1. Left ovary and broad ligament. 2. Right ovary. 3. Fallopian tube. 4. Horn of the uterus. 5. Corpus luteum protruding from ovary. 6. Vagina. 7. Horn of uterus opened. 8. Body of uterus and internal os. 9. External os. Cervical canal between 8 and 9.

II

THE IMPORTANCE OF *BACTERIUM ABORTUM*
OF BANG AND OTHER MICROÖRGANISMS
IN BOVINE INFECTIOUS ABORTION

By JACOB TRAUM

The abortion disease of cattle is rather universally accepted as being caused by an infection. Other agents, nevertheless, have been and are being held responsible for the production of abortion in these animals. Principal among these are mechanical injuries and consumption of moldy, ergotized or otherwise spoiled food. Recently the lack of certain vitamins and failure to maintain the proper mineral balance especially in high-producing cows have been suggested as contributing causes to abortion and other reproductive disturbances. Part II of this bulletin discusses the causes of the infectious type of bovine abortion; the other forms of abortion will be discussed in Part III.

In this, as in other infections, more than one manifestation of disease is produced, and one or more of these may be present; and while in the infection under consideration, the premature expulsion of the fetus is the most outstanding manifestation of its presence, the abortion, nevertheless, does not always occur. This is fully recognized, and practically every definition of bovine infectious abortion,* as the disease is now termed, includes other evidences of the existence of the infection. The report of the committee on abortion of the American Veterinary Medical Association⁵⁹ contains the following definition: "Bovine infectious abortion is characterized as a rule by an interference with the development of the fetus, frequently resulting in its premature expulsion, either dead or alive, viable or unviable. There is also a frequent inflammation of the fetal membranes and of the maternal cotyledons together with frequent retention of the afterbirth. A cow may be the carrier and disseminator of the germs of the disease without showing symptoms of her dangerous condition."

*This disease has been known as contagious, infectious, or epizootic abortion, abortion disease, and slinking of the calves; the name bovine infectious abortion has been suggested by the committee on abortion of the American Veterinary Medical Association.

This, in substance, is the definition of the disease given by most writers on the subject and with few exceptions they also consider that the infectious type of abortion is caused principally by the *Bacterium abortum* of Bang. The above mentioned report states that "The essential cause of bovine infectious abortion is *Bacterium abortus* (Bang)." E. C. Schroeder⁶³, of the U. S. Bureau of Animal Industry, in a paper presented at the Twenty-fifth Annual Meeting of the U. S. Livestock Sanitary Association, states: "The prime etiological factor of bovine infectious abortion was discovered by Bang and Stribolt." Simms and Miller,⁷¹ of the Oregon Experiment Station, in a report of experiments with this disease, state: "The conclusion has been reached that *Bacillus abortus* (Bang) is the principal cause of abortion among Oregon cattle." The term 'infectious abortion' as used in this paper will refer to infection with this organism. Practically all textbooks, such as Huttyra and Marek's "Special Pathology and Therapeutics of Diseases of Domestic Animals," Hoare's "System of Veterinary Medicine," Ostertag and Zwick in Kolle and Wassermann's "Handuch der Pathogenen Microorganismen," accept the Bang organism as the essential cause in infectious abortion of cattle.

On the other hand, Moussu⁵² while admitting that the *Bacterium abortum* of Bang may be responsible for contagious abortion, nevertheless insists that an entirely different bacillus belonging to the paracoli group causes a large proportion of abortions; especially did he find this so in France. W. L. Williams, of the New York State Veterinary College at Cornell University, and some of his colleagues, even though firm believers in the infectious nature of abortion, have persistently refused to accept *Bacterium abortum* as the proved principal cause of infectious abortion in cattle, and a considerable part of Williams' writings are given up to a discussion of the experiments presented as evidence to prove that *Bacterium abortum* of Bang is the principal cause of the infection; and in his latest writings⁸⁴ states: "It has not been determined what, if any, effect upon the reproductive efficiency of a herd the elimination of *Bacterium abortus* infection would cause."

REVIEW OF THE INVESTIGATIONS BEARING ON THE CAUSE OF
INFECTIOUS ABORTION OF CATTLE

To properly discuss and evaluate the results of investigational work which have a bearing on the causative agent of bovine infectious abortion requires the presentation of details. This will be done to the extent that space and importance of the work warrant.

The first report of well-organized attempts to isolate a definite bacterial causative agent of abortion is written by Nocard⁵³, who at the instigation of the French Government investigated this disease in 1885. Nocard gave a very thorough description of the pathological findings, and incriminated as the possible causes a coccus and a short bacillus, which he found in the exudate between the uterus and the chorion, the outer covering of the fetus. With neither of these organisms, however, did he produce the disease.

In 1897, Bang³ reported the results of investigations by himself and Striboldt, in which they discovered the organism now known as *Bacterium abortum*. A cow showing evidence of approaching abortion was purchased and slaughtered. An elastic ligature was tied around the vagina, and the specimen reached the laboratory six hours after slaughter. Using every precaution to eliminate extraneous organisms, they found in the exudate between the uterus and the chorion a very small organism which appeared to be in pure culture. These organisms were found as individuals, also crowded together in masses, and frequently enclosed in cells. The investigators at first thought the organism was a coccus, but on more careful examination with a higher power glass, the bacillary nature of the organism was definitely demonstrated. In shake cultures, consisting of gelatin, agar, and serum, they found a growth developing in a definite zone, beginning about 5 mm. beneath the surface of the medium and extending downward from 10 to 15 mm. They further found that the organism grew also in an atmosphere of very high oxygen tension.

In the course of time they examined twenty-one specimens including pieces of afterbirth, uterine exudate, and aborted fetuses. In almost all cases of uterine exudate, microscopic examination demonstrated the presence of the organism, but always contaminated with other organisms. They succeeded, however, in obtaining pure colonies of the organism by subculturing during the cold time of the year in the specimens received. In three cases where fetuses were sent in, they obtained pure culture of the germ from the small intestines without any evidence of other bacteria. In one case they were able

to obtain the organism also from the blood and in another from the blood, stomach, and *medulla oblongata*. In two cases of mummified fetuses of five and nine months' standing, respectively, they obtained pure cultures of *Bacterium abortum* in each case.

Having repeatedly found *Bacterium abortum*, and having been able to obtain it in pure culture, they next set out to produce the disease in order to definitely demonstrate that it was the cause of infectious abortion in cattle. Four cows bought in the stockyards of Copenhagen were used for this purpose. Two of them received intravaginal inoculations of cultures. After nineteen and twenty-nine days, respectively, these animals showed no evidence of the disease. They were slaughtered and the autopsies were negative. Into the vaginas of the other two cows were placed pieces of retained afterbirth. Thirty-three and thirty-five days afterward, respectively, these animals were slaughtered and autopsy findings again were negative. Bang explained the cause of his failures as being possibly due to the fact that the animals used in the experiment were of unknown history and might have previously aborted and therefore may have been immune. Further, the reason these animals were killed so soon after injection was because the work of Brauer, Lenhert, and Trinchera indicated that the period of incubation for intravaginal inoculation of abortion material is from nine to twenty-one days.

About this time the work of the Committee of the Highland Agricultural Society of Scotland was brought to the notice of Bang through the article on "Epizootic Abortion" by Pemberthy; in this the report of the Committee indicated that the period of incubation with sheep and cattle was from five and one-half to ten weeks. Bang and his co-workers purchased two cows from a territory where abortion was unknown. When these cows were three months pregnant, they were inoculated intravaginally with a culture of the Bang organism. Thirty-nine days later they were again given an intravaginal injection, and twelve days from the second injection they were given a third intravaginal injection of culture. Seventy-one days after the first injection, one of the cows aborted. Six hours after the abortion, the afterbirth was examined and cultured, and from this *Bacterium abortum* was isolated. Cultures and smears also showed the presence of micrococci. The second cow was slaughtered on the same day while showing symptoms of approaching abortion. Abortion bacilli were obtained in pure culture from the exudate. Another cow suffering from pyelonephritis was given an intravaginal inoculation of culture of *Bacterium abortum* on January 19, 1897, and on

March 6 was given a subcutaneous inoculation of culture. Eighty days after the first inoculation a premature but living calf was dropped. The calf died from dysentery at fourteen days. *Bacterium abortum* was obtained in the uterine exudate. Bang concluded that this was a case of uterine catarrh produced by the inoculated organism.

On June 12, 1897, Bang⁴ inoculated 36 c.c. of a bouillon serum culture of the germ intravenously into a four-year-old cow in her fourth month of pregnancy. Three months later this animal aborted and the exudate contained abortion bacilli. This animal, Bang states, came from a farm where abortion did not exist. An eight-year-old cow from an uninfected environment received on June 12, 1897 a culture of *Bacterium abortum* and on September 7, abortion exudate by mouth. On November 26, she gave birth to a small living calf, terminating a pregnancy which started March 14, 1897. The after-birth was covered with abortion exudate, rich in bacilli. Bang states that he was able by intravenous inoculation to produce abortion in cows, sheep, goats, and rabbits.

In 1902, Preisz⁵⁸ of Budapest, Hungary, reported the finding of *Bacterium abortum* in vaginal discharge forwarded to him for examination. In one case the discharge was collected eleven days and in another, fourteen days after abortion. His photograph of the shake culture, the coagulation of the milk, and the successful growing of the organism by the sodium hydroxide-pyrogallie acid method makes the germ a doubtful *Bacterium abortum*. This may account for his inability to produce abortion in two pregnant cattle by intravenous and intravaginal injection. He was also unable to produce abortion in guinea pigs inoculated intravenously, intraperitoneally, subcutaneously, and intravaginally.

In 1908, Nowak⁵⁶ of Austria, reported results of a bacteriological study of the *Bacterium abortum* of Bang, in which he made a valuable contribution, especially in the method of isolating this organism from bacteriological material. In brief, his method consisted of smearing the specimen on agar plate to which had been added one quarter of its volume of sterile serum. This plate was incubated for twenty-four hours; at the end of that time all the colonies were marked and the plates placed in a jar which contained a culture of *Bacillus subtilis*. One square centimeter was provided for every 15 c.c. capacity of the jar. The jar was sealed and placed in the incubator for three days. Material which yielded negative results by the Bang method frequently gave positive results with Nowak's method. By subcutaneous, intravenous, and intraperitoneal injection, Nowak was

able to produce abortion in guinea pigs and in rabbits. By feeding and by intravaginal inoculation, his results were negative.

In 1909, McFadyean and Stockman⁴², reporting for the committee appointed by the Department of Agriculture and Fisheries to inquire into epizootic abortion in Great Britain, gave the results of work started in December, 1905. In the course of their investigations they isolated an organism which satisfied the morphologic and biologic characteristics of *Bacterium abortum*, differing, however, with respect to the optimum growth requirements set forth by Bang. McFadyean and Stockman at times succeeded in growing the organism directly on sloped medium, and in shake cultures the growth was always limited to the zone described by Bang but might appear on the surface and in the water of condensation. Comparisons with cultures furnished them by Bang convinced the English investigators that they had isolated the Bang organism.

In their experimental work they inoculated twenty-eight heifers, as follows: *Intravaginally*: Six heifers with natural material and three with cultures. Of the former, three were positive and three were negative; of the latter, two were positive and one non-pregnant. *Per os*: Four heifers, inoculated with natural material. Two were positive, one negative, and one non-pregnant. One heifer, inoculated with culture *per os*, was positive. *Subcutaneously*: Four, inoculated with natural material. Two were positive and two negative. One animal was inoculated with culture and proved to be positive. *Intravenously*: Two animals were inoculated with natural material and seven with cultures. Of the former, one was positive and one non-pregnant; of the latter, all were positive.

Summarizing the results of these experiments, it is noted that out of sixteen animals inoculated with natural materials, eight were positive, six negative, and two non-pregnant. Of the twelve inoculated with cultures, eleven were positive and one non-pregnant. Of the nineteen positives, seven actually aborted, one gave birth to a weak calf three weeks ahead of time, two were killed while showing signs of approaching abortion, nine were killed at various intervals after inoculation and showed the abortion exudate. In all cases they claimed to have demonstrated the presence of *Bacterium abortum*.

McFadyean and Stockman desired to determine the extent in which this infection was present in England, and in the course of a survey they examined the stomach contents of fifty-one fetuses and found twenty-two positive and twenty-nine negative for *Bacterium abortum*. Thirty-five fetal membranes were examined of which thirty-three were found positive and two negative; the latter two were

reported too putrid for examination. Forty-two swabs of vaginal discharge were examined; twenty-nine were found positive and thirteen negative. The diagnosis in all cases was based entirely upon the microscopic examination of the material.

In 1913, Zwick and Zeller⁹¹ reported the work undertaken by the German Imperial Board of Health on a study of the infectious abortion of cattle in Germany. The report includes work done from December 1908 to December 1911. Their first problem was to determine whether or not the organism described by Bang was responsible for infectious abortion present in Germany. To that end they examined fifty-two aborted fetuses, nine prematurely-born calves, eighteen afterbirths, two uteri, and one vaginal discharge. Of the fifty-two fetuses, thirty-five proved positive for the Bang organism. In thirty out of the thirty-five cases *Bacterium abortum* was obtained in pure culture, but the five other cases were contaminated, two with *Bacterium coli*, two with streptococci, and one with *Bacterium proteus*. Regarding the seventeen negative cases, the investigators report that five had histories which indicated a doubtful presence of infectious abortion. Examination of these five yielded three sterile cases and two which contained *Bacterium coli* in pure and mixed cultures. Cultures from seven other negative fetuses remained sterile although the histories and serum tests from the dams indicated *Bacterium abortum* infection. One fetus yielded sterile cultures although its history indicated infection. Two negative fetuses showed on microscopic examination *Bacterium abortum*-like organisms but the cultures were sterile. One negative case yielded *Bacillus pyogenes*.*

Of the nine prematurely-born calves, two yielded sterile cultures and seven coli organisms, of which six were in pure culture and one with a coccus.

In the eighteen afterbirths examined, the results show five positive for *Bacterium abortum* and thirteen negative for that organism. Four of the positive ones were of mixed cultures and one proved positive after the intravaginal inoculation of sheep. Of the thirteen negative ones, six were badly decomposed and seven showed mixed cultures mostly of *Bacterium coli* and cocci. One uterus from a cow slaughtered before abortion took place yielded organisms of *Bacterium abortum* in pure cultures. The other uterus was from a cow that had aborted four weeks previously and was negative. The one vaginal discharge examined yielded negative results; it was from a cow which had aborted twenty days previously.

* According to Brown and Orcutt⁷ this organism belongs to the corynebacteria, but since this change has not as yet been adopted by the Society of American Bacteriologists, it has not been made here.

Zwick and Zeller confined their animal inoculations to sheep, goats, guinea pigs, and rabbits, and obtained positive results in a very large percentage by introducing the organism into the vagina, subcutis, peritoneum, and mouth.

In twenty-four aborted fetuses, Holth³² obtained cultures of *Bacterium abortum* from the gastro-intestinal contents of nineteen cases. In sixteen of the nineteen, no other organisms were found. Two of the nineteen fetuses yielded cultures of *Bacterium abortum* from the blood. From one of the five cases in which the Bang-organism was not found, a pure culture of *Bacillus pyogenes* was recovered.

In the last decade much literature on infectious abortion of cattle has been published both in America and elsewhere, and some of it contains findings that have an important bearing on the point in question. Prior to that time very little work had been done on the etiology of this disease. Chester¹⁴ of the Delaware Experiment Station reports a study of a herd in which eight cows aborted. From that herd he was able to obtain a piece of placenta, removed by hand, from which he made cultures on agar slants. He obtained one rapidly-growing organism and a second slower-growing one which he identified as the colon organism, and which at that time he thought the possible cause of the disease. A pregnant cow was given 5 c.c. of a bouillon culture of the organism through the vagina and no ill effects resulted.

In 1904, Moore⁵⁰ reported work done by Law and himself in 1897-98, in which they found bacteria belonging to the colon group, in fetal membranes, cotyledons, and uterine discharge in a fresh condition from seven cases of infectious abortion. Other bacteria present to a greater or less extent were bacilli, micrococci, and streptococci. He states, "Its power to produce abortion was tested in a few cases on pregnant cows, all of which delivered their calves at full term. We have no experimental evidence, therefore, that this organism stands in a causal relation to the trouble."

The first investigators to isolate and identify *Bacterium abortum* as being responsible for abortion in cattle in this country were McNeal and Kerr^{43, 44} in 1910. They examined afterbirths and discharges from seventeen cows, ten of which were term deliveries, and in these the abortion organism was not found. Seven were premature deliveries, and of these one was not clinically an abortion case. Two of the six abortion cases were examined before they adopted the Nowak plate method and negative results were obtained. Of the remaining four, two gave positive cultures of *Bacterium abortum*. In the third case only a small bit of the afterbirth was available for examination,

and in the fourth a small quantity of vaginal discharge which had been picked up from the ground. They inoculated four pregnant guinea pigs subcutaneously with culture isolated from these cases; premature evacuation of the uterus resulted, with death of the fetuses after three and a half, eight, six, and seven days, respectively. In the first pig two fetuses were practically fully developed and covered with hair. In this instance *Bacterium abortum* was isolated only from the subcutaneous tissue of the mother at the point of inoculation. In the other three cases, the fetuses were undeveloped and the condition was that of true abortion. In these instances the abortion organism was demonstrated at the point of inoculation, and in the interior of the uterus in pure culture in all three. McNeal and Kerr stated, "From this rather limited series of examinations we may conclude that the bacillus of Bang is the microbic cause of at least some of the contagious abortion of cattle in this country."

In 1912, Good²⁴ of the Kentucky Experiment Station, reported having obtained pure cultures of *Bacterium abortum* from discharges and fetal membranes of aborting cows.

Surface⁸⁰, in 1913, reported the following experimental infection: A cow which had never aborted came from a herd in which abortion did not exist. She had been tested several times by the agglutination and complement fixation tests, always with negative results. When about five months pregnant, she received 20 c.c. of *Bacterium abortum* culture, on November 25, 1912, and aborted on January 6, 1913, fifty-two days after the inoculation. The cultures used were obtained from Denmark and grown aerobically. Cultures were recovered from the afterbirth and fetal stomach in twenty-four hours, both in plates according to the Nowak method, and also in plates kept in the incubator under ordinary atmospheric condition. This very rapid growth under ordinary atmospheric condition is given by Surface as additional proof that he produced abortion with the culture with which he inoculated.

Moore and Fitch⁵¹ in 1911 inoculated five pregnant cows intravenously with cultures of *Bacterium abortum*. Abortion took place in all five at the end of seven, twenty-six, sixty-five, eighty-five, and eighty-five days, respectively, after inoculation. Characteristic lesions of abortion were present in every instance except in the first animal. This experiment is of doubtful value since two of these five cows had aborted, two had been sterile, and but one had calved the preceding year. In 1912, four more cows, which so far as known had never aborted, were fed cultures of *Bacterium abortum*. Three of these animals aborted and one had retained afterbirth. Serum tests

were not made before feeding these animals. All of them, however, showed an increase in agglutinins after feeding, and two of the aborters, which were definitely negative on the first two tests, after feeding, developed an agglutination of 1 to 600. In 1913, four more cows, were fed abortion organisms. There was a rise of agglutinins following the feeding in all cases. Three were still pregnant at the time of the writing of the report. One dropped a weak calf at 263 days of pregnancy.

In 1913 Mohler and Traum⁴⁹ reported the finding of *Bacterium abortum* in eleven out of fifteen aborted fetuses.

W. L. Williams⁸⁵, in 1914, reported experiments with six heifers raised carefully under his direction. These he infected by intravenous inoculation of a culture of the same strain as used by Moore and Fitch. One of the six died of tuberculosis. Five others were killed from three and a half to six months after inoculation, respectively. Four were normal and no abortion organisms were found; the fifth showed in the utero-chorionic space abortion exudate from which *Bacterium abortum* was isolated.

Another lot of three animals⁸⁶ were inoculated intravenously. One of these aborted, one calved at the end of 272 days but had retained afterbirth and metritis; the third calved at the end of 284 days with prompt expulsion of the fetus but metritis developed nine days after parturition. In another group of three heifers, two were given cultures by the mouth. One of them which reacted strongly before the culture was given aborted. The other calved normally.

Following the recording of the above experiments of Moore and Fitch and of his own, Williams states: "With full regard for the defects in the evidence, it is nevertheless extensive and important, and justifies amply the general conclusion that the Bang organism is the essential cause of contagious abortion, a conclusion freely open to revision whenever new evidence may demand."

In the reports of the New York State Veterinary College for years 1917-18⁸⁷ and 1918-19,⁸⁸ published in 1919 and 1920, respectively, Williams reviews the work of Nocard, Bang, McFadyean and Stockman, Moore and Fitch, his own experiments just mentioned, and the report of Bland, in which the latter gives a record of inoculating five pregnant cows and nine pregnant heifers by intravenous inoculation for the purpose of immunization (inoculation by mistake since Bland thought these animals were not pregnant). Of these fourteen animals, only two cows and one heifer aborted. Williams also refers to the cultures made by Day of the United States Bureau of Animal Industry at Chicago. From the uterine cavity of twenty-five preg-

nant cows, seventeen showed no growth; two, streptococci and short bacilli; three, staphylococci and short bacilli; one, streptococci and staphylococci. In a similar work done by Hagan at the Buffalo stock yards, in which he examined twenty-six uteri, sixteen of which were pregnant, he found two sterile, seven which yielded coli-like organisms, one micrococci, three streptococci, three streptococci and staphylococci, and in one of these there was also found paracolon. Two out of the sixteen showed exudation in the utero-chorionic space; in one a micrococcus was found and in the other a streptococcus. It appears that Williams lays too much stress upon the findings by Day and Hagan of the various organisms in pregnant and non-pregnant uteri and attributes to these organisms an unproved importance in cattle abortion.

In 1918 Theobald Smith⁷² reported the finding of spirilla or vibrios in pure culture from the fetuses of fourteen cases of abortion. He states that the condition of the fetus is much the same whether spirilla or bacteria of abortion are present, and that changes in the chorion due to vibrio cannot be distinguished with the naked eye from those due to the organism of Bang⁷³. In 1919, Smith⁷⁴ reported the finding of twelve more cases of spirilla infection in aborted fetuses. One of these twelve was associated with *Bacterium abortum*. In the grouping of cases of abortion according to the associated bacteria, in one herd Smith⁷⁵ recorded sixty-two cases from which *Bacterium abortum* was isolated, twenty-six cases of pure spirilla infection (one of the spirilla cases was associated with *Bacterium abortum* infection); another group of twenty-one abortions from which neither *Bacterium abortum* nor *Vibrio fetus** was isolated—Smith considers that one or two of the twenty-one cases may possibly belong to the group of *Bacterium abortum* infections and several may come within the group of *Vibrio fetus*. Two others were cases of infection with *Bacillus pyogenes*, in which the preceding pregnancy had been cut short by *Bacterium abortum* infection. In eight cases miscellaneous rapidly-growing bacteria appeared in the cultures. Of these several may be regarded as cases in which bacteria gained entrance after expulsion, the fetus being large enough to live a short time after birth. One was clearly a case of asphyxiation at birth and one was regarded by the attendant as traceable to fighting several days before expulsion of the fetus. Seven were considered bacteria-free. Smith states, "How many of these seven are due to injury or toxic substance and food factors cannot be stated."

*Name suggested by Smith for the spirillum isolated from abortion cases and accepted by the Committee of the Society of American Bacteriologists on Characterization and Classification of Bacterial Types.

Smith⁷⁴ succeeded in producing a disease of the fetal membrane and finding the vibrios in two out of four pregnant cows, injected intravenously with cultures of his *Vibrio fetus*. *Bacterium abortum* infection was ruled out in these cases. Among the twenty-six cases associated with *Vibrio fetus* of which one also contained *Bacterium abortum*, there were no heifers involved. All were second or later pregnancies. Recently, however, Smith, Little, and Taylor⁷⁷ found three cases of abortion in native heifers in the same herd associated with *Vibrio fetus*.

Vibrios have been recovered from abortions in ewes in the British Isles by McFadyean and Stockman⁴¹ and in this country by C. M. Carpenter⁹. The former also report the finding of vibrios in cattle abortion in Ireland and in Wales. *Bacterium abortum* was absent in these cases.

In the report of the New York State Veterinary College for the year 1920-21, C. M. Carpenter¹⁰ recorded the bacteriological studies of cattle genital tracts and fetuses. Among these are included the findings in eleven pregnant apparently normal uteri. In these he found six uteri sterile; *Bacterium abortum* was found in fetal fluids in one case; three uteri showed streptococci, one *Pseudomonas pyocyaneus*, and one showed an unidentified rod. Cultures from eighteen normal non-pregnant uteri yielded no growth in seven uteri, four showed staphylococci, and three streptococci in the cultures. Three developed streptococci and staphylococci, and one *Pseudomonas pyocyaneus* in the cultures.

In twenty-three fetuses from apparently normal pregnant uteri, Carpenter obtained ten negatives, three showed streptococci alone, three staphylococci alone, and three streptococci and staphylococci together. Three showed *Bacterium abortum* in pure culture in the stomach; one of the three showed no other organism; in another streptococci were found in the meconium of the rectum.

Bacteriological examination of twenty-five aborted fetuses yielded the following results: *Bacterium abortum* was found in ten cases. In nine it was found in pure culture, eight times in the stomach alone or also in other organs, and once in the genital tract. In five of these nine cases, no other organism was found in the fetus. In the tenth case staphylococci and *Bacterium abortum* were found in the heart's blood. Streptococci alone were found in two cases. In two other fetuses streptococci associated with other organisms were obtained. In four fetuses staphylococci alone or associated with other organisms were obtained. In one case a Gram positive rod, and in another a Gram positive spore-bearing rod was found.

Carpenter also made a bacteriological examination of three mummified fetuses. In one he found *Streptococcus viridans*. In the other, two organisms from the colon-aerogenes group were obtained. One of the latter cases was associated with *Bacterium abortum*.

Rettger, White, and Chapman⁶⁰ in a recent publication reported results of their attempt to produce abortion by artificial inoculation of cultures of *Bacterium abortum*. In their first trials where fourteen feedings of *Bacterium abortum* in capsules were given to eight cattle between the ages of two to twelve years, they obtained uniformly negative results. In the second attempt ten calves were fed cultures in pasteurized milk and after weaning were given cultures in capsules. Here again they obtained negative results. In both experiments the feeding of the organism in capsules did not incite the production of agglutinins or complement fixing bodies. Ten calves were used as controls. One of these became a confirmed reactor to the serologic tests and aborted, another aborted later.

In another experiment three heifers were injected subcutaneously with culture of *Bacterium abortum*. One aborted and the others gave positive reactions. The inner surface of the vulvas of three other heifers was scarified and coated with cultures of *Bacterium abortum*. One of these gave a positive reaction and aborted five months after infection. The other two remained negative to the test, one calving normally and the other found to be non-pregnant. Eight heifers received superficial intra-urethral injection of cultures of *Bacterium abortum*. Six became reactors to the serological test. One gave only partial reactions and one was negative. Two of the reactors dropped premature small but live calves. In a further experiment, seven animals received deep intravaginal injection of *Bacterium abortum*. Three of the seven became confirmed reactors, two aborted, another gave birth to a small calf and a third calved normally. The other four resisted injection and remained negative to the serological tests.

Huddelson³³, in his studies on artificial immunization against bovine infectious abortion used fifteen heifers, none of which had ever given a positive blood test, nor had they ever so far as known been subjected to abortion infection before this experiment. Six were immunized, four were kept as normal controls, five were controls on the mode of infection after immunization and conception. Three of these five received intravenous injection of 1 c.c. of *Bacterium abortum* and two were fed living cultures of *Bacterium abortum*. Three of the normal controls calved normally and one failed to conceive. Each of the five infected controls, however, aborted about ten weeks subsequent to the appearance of antibodies in the blood serum.

Seddon⁶⁸ in his studies on abortion diseases records the infection of a cow by drenching on the sixty-second day of her pregnancy, which resulted in premature calving on the 262nd day and the finding of *Bacterium abortum* in the uterine exudate. Another animal which was injected subcutaneously with *Bacterium abortum* aborted eighty-two days after infection, on the 249th day of pregnancy. The uterine exudate contained abortion bacilli (cultures and guinea pig inoculations). A third heifer on the sixty-third day of gestation received intravenously, living *Bacterium abortum*, and 108 days after the inoculation the animal aborted. Organisms were found in the fetus and in the exudate. A fourth heifer when pregnant 225 days received abortion organisms in the vagina. She calved the 268th day of pregnancy, which was sixty-seven days after the infection. The uterine exudate contained *Bacterium abortum*. He does not state in his article the number of animals, if any, which were negative to the inoculation.

Sven Wall's⁸³ extensive studies on alterations in the uteri in abortion and chronic metritis are pertinent to our discussion. His findings were as follows: In two gravid uteri showing metritis, chorionitis, and placentitis, *Bacterium abortum* alone was found in the exudate. In another gravid uterus containing odorless thick pus in the utero-chorionic space, *Bacillus pyogenes* alone was found; this case showed chorionitis and metritis, but no placentitis. In a fourth gravid uterus, between the unaltered fetal membranes and the red-colored, partly denuded uterine mucosa, about fifteen cubic centimeters of yellow, odorless, slimy pus were observed lying at the internal opening of the cervical canal. Smears showed streptococci and probably *Bacterium coli*. This case was two days old when examined.

In two uteri, containing mummified fetuses, *Bacterium abortum* alone was found. From four other cases of uteri containing mummified fetuses Wall obtained from one, *Bacillus pyogenes*, from another *Bacillus pyogenes* and a streptococcus, from the third case a streptococcus, and from the fourth a streptococcus, *Bacterium coli* and a diphtheroid.

From the uterine cavity of two cattle that came to autopsy some days after abortion, he isolated *Bacterium abortum*. In one case this organism was associated with streptococci and in the other with *Bacillus pyogenes* and *Bacterium coli*.

In three cases of retained afterbirth and in fifteen cases of chronic metritis he found, as a rule, either streptococci or *Bacillus pyogenes* or both, and in some instances these were complicated with *Bacterium coli* or *Bacterium proteus* putrefaction.

PATHOGENICITY TESTS OF VARIOUS STRAINS OF BACTERIUM
ABORTUM

Since the work of Smith and Fabyan⁷⁹ and that of Schroeder and Cotton⁶⁶ upon production of the disease in guinea pigs by inoculation of *Bacterium abortum*, these experimental animals have been used a great deal for determining the presence and the isolation of *Bacterium abortum* from suspected materials. They have also been used for the study of comparative pathogenicity of various strains of *Bacterium abortum*.

Schroeder⁶⁴ reports that, together with W. E. Cotton, he studied three porcine strains, one from Indiana, the second from Illinois, and the third from California. They found these strains to differ in their pathogenicity for guinea pigs. They were not wholly like any of the numerous other bovine strains studied by them. The lesions produced by porcine strains in guinea pigs were grosser in character, bone lesions were commoner, and post-orbital abscesses developed. One of the porcine strains produced abortion in cattle and sows.

From a comparative study of ten porcine strains from three outbreaks in California and of five bovine strains of *Bacterium abortum*, the writer⁸² concluded: "Porcine strains, on the whole, proved to be more virulent for guinea pigs, causing, with few exceptions involvement of the testes and one or both radio-carpal regions, also general adenitis. Thirteen of the twenty-two guinea pigs inoculated with porcine strains succumbed within two months, while none of the twelve inoculated with bovine strains died of infection. The dose for each guinea pig in the above series was similar."

Meyer, Fleischner, and Shaw⁴⁶, in their study on the pathogenicity of *Bacterium melitensis* and *Bacterium abortum* for guinea pigs, review the work done and results obtained in guinea pig inoculation with *Bacterium abortum* by Smith and Fadyan, Schroeder and Cotton, T. C. Evans, Krage, Thomsen, Holth, Robinson and Zeller. From their review, it can readily be seen that the lesions in guinea pigs have not been found by many of the above mentioned workers.

Meyer, Fleischner and Shaw studied the pathogenicity of four bovine strains upon twenty-two guinea pigs; eighteen were inoculated intratesticularly and four subcutaneously. Two strains were pathogenic for guinea pigs in fifteen out of sixteen cases, and the other two strains produced lesions and infection in one each of three inoculated intratesticularly with each strain. One of their pathogenic strains which had been isolated from milk, was two years old. The history of the other strain is unknown. The dose used in the above inoculations was from 1000 to 2000 million viable organisms. They

conclude that until knowledge relative to individual susceptibility of the guinea pig has been accumulated, it is impossible to make conclusive statements, but the few, carefully-controlled observations rather forcibly suggest that old stock cultures are not only less toxic but less invasive than recently isolated strains or infected material derived from bovine, porcine, or avian sources.

These investigators also found *Bacterium abortum* from sows highly virulent for guinea pigs. Six out of twenty-four animals succumbed in from seventeen to forty days after intraperitoneal inoculation. The strain exhibited pyogenic properties. The testicles, epididymes, and seminal vesicles of all the animals used in the tests were destroyed by suppurative processes. The spleen was usually enlarged. Nine guinea pigs showed involvement of the ribs and long bones, while eleven showed lesions only in the radio-carpal and tarsotibial regions. They also report in tabulated form the lesions found in 114 guinea pigs inoculated with stock cultures, milk and guinea pig material at various periods after inoculations.

Huddleson³⁴, in his recent study on comparative pathogenicity of several strains of *Bacterium abortum*, used twenty-one strains on one hundred and twelve guinea pigs. From his work, one can conclude in a general way that older cultures lose their virulence for guinea pigs. In fact, he states: "A majority of strains isolated became non-virulent within one year after isolation." A study of his results indicates that there appears to be a difference of the individual strain irrespective of time of its isolation. For instance, his strains 20 and 805 were both isolated in May, 1919; the former uniformly produced lesions while the latter was uniformly negative. His strain 16 yielded no-lesion guinea pigs in January, 1920 and August, 1921, but was positive April, 1921. One swine culture failed to produce lesions in guinea pigs, while another strain from Illinois yielded positive and negative guinea pigs.

OBSERVATIONS UPON FORTY ABORTED FETUSES RECEIVED AT BERKELEY

Agglutination tests of blood sera from cattle in a number of herds in various parts of California indicated that abortion was in a large percentage of cases due to *Bacterium abortum* infection; nevertheless it was deemed advisable to make a bacteriological study of aborted fetuses from dairies within a radius of one hundred miles of Berkeley. In general, the procedure consisted in making three cultures from the stomach contents on cooked blood agar, fetus liver agar, 2 per cent glycerol and 1 per cent glucose agar, respectively, three on the same kind of media from the lungs and one from the meconium,

and placing these under CO_2 conditions, as suggested by Huddleson.³⁵ Plates were streaked with stomach contents and meconium from the rectum and kept under ordinary atmospheric conditions in the incubator. Figure 2* illustrates the method used in our laboratory to obtain the recommended CO_2 conditions. Fifteen per cent of the air is drawn off by suction and replaced by 10 per cent of carbon dioxide from the tank. It was found necessary to remove 5 per cent more air than was replaced by gas to allow for expansion when placed in the incubator.

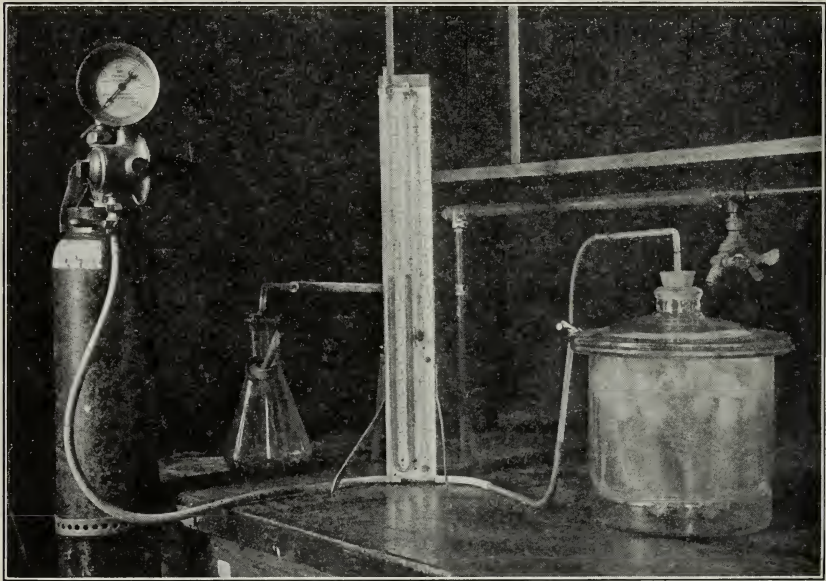


Fig. 2.—Illustrating Method Used in Growing *Bacterium abortum* Under CO_2 .

The Pressure in the desiccator is reduced by suction until manometer reads 144 mm. (marked 15%). CO_2 is allowed to flow in from cylinder until pressure reads 38 mm. (marked 5%). Upon heating to 37°C . in incubator, the pressure will again become approximately atmospheric.

Besides inoculating the cultures, one guinea pig was inoculated intraperitoneally with stomach contents and another with lung extract. Hanging drop preparations were made at the time of autopsy, with a special view to determining the presence or absence of spirilla. Smears were made from the stomach contents and lung in duplicate and were stained with Gram and other methods as it was thought desirable. Table 1 contains the result of such examination upon forty fetuses made by Hart and the writer.

*The modification of the Huddleson method, as illustrated in Figure 2, was suggested by H. Goss of the Nutrition Division. The manometer was also made by him.

TABLE I. BACTERIOLOGICAL FINDINGS IN ABORTED FETUSES

NOTE.—In culture column in chart + indicates *Bacterium abortum* found; — indicates no *Bacterium abortum* found and if other organisms were found that fact is mentioned alongside of the —. Each + or — represents a tube of medium. In last column + indicates *Bacterium abortum* demonstrated in that case, — indicates *Bacterium abortum* not demonstrated in that case. * Smears from stomach contents showed vibrio with morphology like these described by Smith. † A spore-bearing organism found in smears from lung. Probably anaerobe.

No.	Date Received	How	Approximate Age	Cultures			Guinea Pig Inoculations	Remarks	
				Stomach	Lung	Meconium Rectum			
1	Oct. 26, 1921	Truck		+ + — Contaminated	+ + —	Not made	Stomach Lung	+ +	
2	Dec. 13, 1921	Messenger	5 Mo.	— — — Gram negative acid producing rod	— — — Contaminated	Not made	Stomach Lung	— — —	Evidence and history indicates cervix open several days before expulsion
3	Dec. 16, 1921	Truck	5 Mo.	+ — —	+ + +	Not made	Stomach Lung	+ —	+ —
4	Dec. 27, 1921	Messenger	4 Mo.	— — — colon	— — —	Not made	Stomach Lung	— —	Partially mummified
5	Jan. 7, 1922	Truck		— — —	— — —	Not made	Stomach Lung	+ +	+ +

TABLE 1. BACTERIOLOGICAL FINDINGS IN ABORTED FETUSES—(Continued)

No.	Date Received	How	Approximate Age	Cultures			Guinea Pig Inoculations	Remarks
				Stomach	Lung	Meconium Rectum		
6	Jan. 9, 1922	Truck	7 Mo.	-	-	Not made	Stomach Lung	-
				-	-			
				-	-			
7	Jan. 11, 1922	Messenger	Mature	-	-	Not made	Stomach Lung	+ + +
				-	-	Overgrown		
				-	-			
8*	Jan. 16, 1922	Truck	8 Mo.	-	-	Not made	Stomach Lung	-
				-	-			
				-	-			
9	Jan. 18, 1922	Truck	7½ Mo.	-	Pyogenes colonies	Not made	Stomach Lung	- - -
				-				
				-				
10	Jan. 25, 1922	Truck	7 Mo.	+	-	+	Stomach Lung	+ +
				+	+			
				+	+			

TABLE 1. BACTERIOLOGICAL FINDINGS IN ABORTED FETUSES—(Continued)

No.	Date Received	How	Approximate Age	Cultures			Guinea Pig Inoculations	Remarks
				Stomach	Lung	Meconium Rectum		
11	Jan. 25, 1922	Truck	7 Mo.	+ + +	+ + +	+	Stomach Lung	+ +
12	Feb. 1, 1922	Express	7 Mo.	+ - -	+ + +	-	Stomach Lung	+ -
13	Feb. 3, 1922	Truck	8 Mo.	+ + -	+ + -	+	Stomach Lung	+ +
14	Feb. 6, 1922	Truck	7 Mo.	- - -	+ and unidentified organism - =	-	Stomach Lung	+ +
15	Feb. 7, 1922	Truck	8 Mo.	+ - -	+ - -	-	Stomach Lung	+ +
16	Feb. 19, 1922	Truck	Almost at term	- - -	- - -	-	Stomach Lung	- -

TABLE 1. BACTERIOLOGICAL FINDINGS IN ABORTED FETUSES—(Continued)

No.	Date Received	How	Approximate Age	Cultures			Guinea Pig Inoculations	Remarks
				Stomach	Lung	Mecconium Rectum		
17	Mar. 5, 1922	Truck	Almost at 4 Mo.	- - -	- <i>Bacillus subtilis</i>	-	Stomach Lung	- -
18	Mar. 10, 1922	Express	Breathed	+ and 4 con- taminating colonies	+ and con- taminating colony + and con- taminating colony - mold	+	Stomach Lung Placenta	+ + +
19	Mar. 15, 1922	Truck	7 Mo.	- - -	- con- taminated - con- taminated	Staphyloco- ccus aureus	Stomach Lung	- -
20	Mar. 17, 1922	Truck	7 Mo.	+ + +		+	Stomach Lung	+ +
21†	Mar. 22, 1922	Express	5 to 6 Mo.	- - -		-	Stomach Lung	- -

TABLE 1. BACTERIOLOGICAL FINDINGS IN ABORTED FETUSES—(Continued)

No.	Date Received	How	Approximate Age	Cultures			Guinea Pig Inoculations	Remarks
				Stomach	Lung	Meconium Rectum		
22	Mar. 21, 1922	Express	Fully developed	— — B. pyogenes — B. pyogenes	— — B. pyogenes — B. pyogenes	—	Stomach — Lung —	Pyogenes infection
23	Mar. 23, 1922	Truck	8 Mo.	— — —	— — —	—	Stomach — Lung —	—
24	Mar. 30, 1922	Express	4 Mo.	— unidentified — unidentified — unidentified	— — —	—	Stomach — Lung — Placenta —	—
25	April 3, 1922	Truck	8 Mo.	+ + —	+ + +	—	Stomach + Lung +	+
26	April 5, 1922	Express	Nearly mature	— Unidentified organism — Unidentified organism — Unidentified organism	— — —	—	Stomach — Lung —	—

TABLE 1. BACTERIOLOGICAL FINDINGS IN ABORTED FETUSES—(Continued)

No.	Date Received	How	Approximate Age	Cultures			Guinea Pig Inoculations	Remarks
				Stomach	Lung	Meconium Rectum		
27	April 12, 1922	Express	Full term	— Colon group — Colon group — Colon group	— Colon group — Colon group — Colon group	—	Stomach, dead April 14, 1922. Lung —	Badly decomposed
28	April 17, 1922	Express	Full Term	Cultures all overgrown variety of organisms			Stomach, pig dead April 18, 1922 Lung —	Badly decomposed, from same place as 27.
29	April 28, 1922	Express	4 to 5 Mo.	None Made	— Overgrown — —	—	Lung — Placenta +	Cultures made from lung. Abdominal viscera not available.
30	May 3, 1922	Messenger	5 Mo.	— — —	— — —	—	Stomach — Lung —	—
31	May 12, 1922	Express	7 to 8 Mo.	— Staphylo- cocci, colon and another rod — —	— Staphylo- cocci, colon and another rod — —	—	Stomach, dead May 13, 1922 Lung —	—

TABLE 1. BACTERIOLOGICAL FINDINGS IN ABORTED FETUSES—(Continued)

No.	Date Received	How	Approximate Age	Cultures			Guinea Pig Inoculations	Remarks
				Stomach	Lung	Meconium Rectum		
32	May 13, 1922	Truck	9 Mo.	-	+		Stomach Lung	Portion of lung filled with air +
				-	+	-		
				-	+			
33	May 20, 1922	Messenger	7 Mo.	+	+		Stomach Lung	+
				-	-	+		
				-	-			
34	May 24, 1922	Express	4 to 5 Mo.	-	-		Stomach Lung Placenta	-
				-	-			
				-	-			
35	May 25, 1922	Truck	8 Mo.	-	+		Stomach Lung	+
				-	-	-		
				-	-			
36	June 1, 1922	Express	8 Mo.	-	-		Stomach Epicardium	-
				-	-			
				-	-			

TABLE 1. BACTERIOLOGICAL FINDINGS IN ABORTED FETUSES—(Concluded)

No.	Date Received	How	Approximate Age	Cultures			Guinea Pig Inoculations	Remarks
				Stomach	Lung	Meconium Rectum		
37	June 1, 1922	Truck	Full term	- - +	Staphylococci and colon and Staphylococci and colon	+ + -	Stomach Lung	Lungs showed evidence of having been filled with air +
38	June 12, 1922	Express	8 Mo.	+ + -	and 2 con-taminating organisms	- + -	Stomach Lung	+ + +
39	June 14, 1922	Express	8 Mo.	- - -	coli and coccus	- - -	Stomach Lung	- -
40	June 15, 1922	Truck	5 Mo.	+ + +		- + -	Stomach Lung	+ +

Table 1 needs little comment. In the third column the means by which the fetuses reached our laboratory are indicated. This was included since we found that fetuses sent in by express did not arrive in as good condition as those received by truck or messenger. The truck bringing milk from the valley would pick up properly packed fetuses and deliver them to us, as a rule, within five hours after being received by the truckman. Of course, some fetuses had been dropped some time before the veterinarian received them, but the veterinarians living along that truck line were impressed with the importance of obtaining fetuses in good condition. The fetuses received by truck or messenger were on the whole either positive for *Bacterium abortum* or else sterile; especially was this true during cold weather. In the cases where there was evidence of lung expansion by air, there were always contaminating organisms.

In cases negative for *Bacterium abortum*, it was not always possible to obtain as much history as desirable; nevertheless, some was obtained and deserves mention here. Cases 2 and 4 came from the same dairy, and an agglutination test of sera from eight aborting cows several weeks after abortion gave four strong positives, one partial, and three negatives. Sera from the dams of the two fetuses were not obtained. Fetus 8 came from a cow which was the only animal to abort in a herd of eight. Serum from the aborting cow, obtained April 26 gave negative agglutination test. Six other cows in this herd were also negative; one cow gave positive reaction in .02 c.c. serum but was negative in .01 c.c. to two antigens. A strong positive was obtained in one animal added to the herd after the abortion occurred. Regarding the aborting cow, the owner informed us that she threw herself in the stanchion on an uneven wooden floor and three days later aborted fetus 8. (This case mentioned in article III under injury). Notations above Table 1 indicate that vibrios were found in smears from the stomach contents.

Fetus 9 was obtained from a herd of twenty of which two aborted; one of the aborted fetuses was our No. 9. All but the two aborters were sold. Agglutination test upon serum drawn April 27, 1922, indicated that the dam of fetus 9 was a strong reactor and the other aborter showed positive reaction in .04 c.c., and partial in .02 c.c.; but negative in .01 c.c. From fetus 9 *Bacillus pyogenes* alone was isolated from stomach and lung. Smears from these organs showed enormous numbers of a Gram positive pleomorphic organism which proved to be *Bacillus pyogenes*.

Fetuses 21, 22, 24, and 26 came from a dairy of over 200 animals where several years ago a large number of serum tests were made,

which resulted in finding many cases positive for *Bacterium abortum*. Fetuses 24 and 26 exhibited a similar but as yet unidentified organism in the stomach contents of each. A study of abortion in this herd is being made. In case 22 nothing but *Bacillus pyogenes* was obtained.

Fetuses 27 and 28 forwarded by the same man reached the laboratory in a very bad state of decomposition. Cultures were overgrown and the guinea pig inoculation from lung alone remained for diagnostic purposes in each case.

Fetus 36 was one of twins and was dropped by a cow that three days previously had given birth to an eight-month but apparently normal, dead fetus. (We did not see this fetus). Our autopsy on Fetus 36 is as follows: 100 cm. long, skin and hair apparently normal; no union of epiphyses and diaphyses, bones of skull easily and freely movable, ribs unattached, bones of vertebral column and their processes separated, gas in subcutis and pleural cavity. Fibrinous exudate presenting roughened appearance present on the thoracic and abdominal walls; same roughened appearance on heart and pericardium. Muscle of legs greatly shrunken.

Regarding the other fetuses negative for *Bacterium abortum*, there is nothing to be added to what is shown in Table 1.

DISCUSSION

In the introductory pages it was pointed out that even though the disease is termed "Bovine Infectious Abortion," and even though abortion is the most prominent symptom, it is not necessary for the premature expulsion of the fetus to occur in order to give evidence of the existence of an infection that can and does frequently produce abortion. In estimating the power of any organism to incite the production of one or more of the changes in the pregnant uterus and its fetus which frequently bring about abortion, we must not insist upon the occurrence of this manifestation, but may safely accept an organism as being the cause of abortion when it has been demonstrated that such organism is capable of locating itself in the pregnant uterus and setting up inflammatory changes there which involve the placenta and chorion and which often manifest themselves clinically by an endometritis and retained afterbirth. Such inflammatory processes may and often do proceed to such an extent as to infect the fetus or interfere with its proper development, resulting in the discharging of a weak or dead fetus at various stages of gestation. Sometimes the fetus perishes but is not expelled and becomes mummified.

The Power of Bacterium Abortum to Produce Abortion Disease Manifestations.—That *Bacterium abortum* has such power is amply indicated by the experimental evidence quoted above. Bang demonstrated that fact, not only by finding the organism in the exudate in the utero-chorionic space and in the aborted fetuses, but also in practically all of his experiments with cattle excepting the first four cows inoculated by him which were slaughtered soon after inoculation. McFadyean and Stockman, by causing the production of inflammatory changes and the resultant abortion exudate, or by causing the actual premature expulsion of the fetus, and by the demonstration of *Bacterium abortum* in the exudate and fetus in their experimentally-inoculated heifers, showed that *Bacterium abortum* satisfies the requirements set forth above. They made this demonstration with eight out of fourteen pregnant cows inoculated with natural material and in all of eleven cases inoculated in various ways with cultures of *Bacterium abortum*.

Sven Wall added further evidence in this respect when in two gravid uteri he found inflammatory changes involving the chorion, endometrium, and placenta, and in the utero-chorionic exudate he found only *Bacterium abortum*. The recovery of *Bacterium abortum* alone in two mummified fetuses may also be included as support of the power of this organism to cause fetal death.

Moore and Fitch's report indicates that at least in their second infection experiment, they were able to bring about abortion by feeding cultures of *Bacterium abortum* to pregnant cows.

The work of Huddleson, Seddon, and others, who infected non-infected cattle with *Bacterium abortum* and produced abortion or exudative inflammation of the uterus and chorion in which the inoculated organism was found is added proof that *Bacterium abortum* is concerned in the production of bovine infectious abortion.

The investigations of E. M. Robinson^{61, 62}, in which he claims to have eliminated infectious abortion through application of the agglutination test with *Bacterium abortum*, add to the evidence that *Bacterium abortum* is able to produce abortion. After four years' experience with this method he still recommends it. Simms and Miller also claim that testing all cattle and eliminating reactors will certainly tend to control and possibly eradicate abortion. Details of these observations are fully discussed in the paper on "Methods of Controlling Abortion."

The Failures of Bacterium Abortum to Produce Abortion Disease Manifestations.—In some of the experiments with *Bacterium abortum* quoted in the preceding pages, it was not possible to produce actual

abortion or changes in the uterus and chorion in a large percentage of inoculated animals. Williams succeeded in only one out of five heifers in his first inoculation experiment. He had a higher percentage of successes in his two other attempts upon five animals. Bland, quoted by Williams, succeeded in producing abortion by inoculation of *Bacterium abortum* in only three out of fourteen cases. Rettger, White, and Chapman failed to infect eighteen heifers fed *Bacterium abortum* in capsules. Their experiments were rather unusual on account of the fact that several animals were fed cultures before or at the time of and at various periods after breeding. They caused infection by inoculating the culture into the subcutis, into the vagina upon the urethra and upon the scarified vulva. In view of the successful infections via the digestive tract by other observers, it can only be concluded from the work of Rettger, White and Chapman that they were unable to infect cattle by introducing into the mouth capsules containing *Bacterium abortum*.

The review of the work on the pathogenicity of *Bacterium abortum* given above indicates that there is a difference of virulence for guinea pigs in different or even in the same strain of this organism, and failures to produce the well-established inoculation disease in guinea pigs with *Bacterium abortum* explain to some extent the failure to produce bovine infectious abortion sometimes in small and sometimes in large percentages of experimentally-inoculated animals.

Extent of Bacterium Abortum Infection.—Reports of the investigation upon this disease as reviewed above yield some information as to the extent in which abortion has been caused by *Bacterium abortum*. The British Commission's data along this line, even though based entirely upon microscopic examination, and therefore not altogether reliable, nevertheless deserves recapitulation here. Out of fifty-one fetuses examined, twenty-one were pronounced positive for *Bacterium abortum*, and twenty-nine were negative. Out of thirty-five fetal membranes, thirty-three were positive. Of forty-two vaginal swabs twenty-nine were found to be positive. In this connection it might be stated that it has been our experience that the microscopic examination of smears from the stomach contents and lung tissue from the aborted fetuses frequently gave negative results for *Bacterium abortum* when cultures or guinea pig inoculation, or both, of the same material proved to be positive.

More satisfactory estimates of the extent of the *Bacterium abortum* infection in cases of abortion may be made by referring to the results obtained by the various investigators who made cultural or guinea pig inoculations, or both, of materials obtained from the aborted

fetuses. Here we find the percentage of cases where *Bacterium abortum* was found to vary from 40, in the work of C. M. Carpenter, to 79.1 as reported by Holth. Without overestimating the importance of *Bacterium abortum*, it should be stated that this organism might be responsible for abortion even if it cannot be demonstrated by cultural and guinea-pig inoculations of fetal material alone. In a good many of the reported cases placental tissue was not available.

In a fair percentage of the fetuses negative to *Bacterium abortum*, no organisms of any kind could be demonstrated. Thus Zwick and Zeller found thirteen out of fifty-two fetuses sterile upon cultural inoculations. Smith reported seven out of one hundred nine cases as sterile, and eight fetuses showed rapidly-growing organisms which he considered as probably having gained entrance after the fetus had been dropped. Carpenter found two out of twenty-five that yielded no organisms.

Table 1 shows that our cases 16, 17, 23, 30, and 34 yielded no growth in our cultures and inoculations. In our fetuses 2, 4, 6, 19, 21, and 39, the extent and the character of the growth found suggests strongly unimportant contamination. Our cases 27, 28, 31, and 36 were decomposed to such an extent as to expect the finding of the organisms indicated in the table.

Other Organisms Connected With Abortion Disease Manifestations.—Of the microorganisms other than *Bacterium abortum* that have been associated with the infectious type of abortion, the spirillum or *Vibrio fetus*, commands first attention, principally on account of the work of Theobald Smith. In one herd of one hundred nine abortions he found twenty-six cases of abortion* associated with *Vibrio fetus* and in twenty-five of these *Bacterium abortum* could not be demonstrated. He further incriminated the vibrio by producing disease of the fetal membranes in two out of four cows injected with the vibrio. Judging from the literature on cattle abortion, this infection must be extremely rare. Smith's report of these findings in one herd is practically the only report of bovine abortion in America attributed to this infection. The British Commission mentions the finding of vibrio abortion in cattle in one outbreak in Ireland and in another in Wales.

In fetus 8 we demonstrated the presence of vibrios in the stomach contents. The dam of this fetus gave negative agglutination test with *Bacterium abortum* antigen. In this case there is also a history of injury, the importance of which was not determined.

*The review of literature above shows that three more cases of vibrio infection were found in this herd after Smith had reported a grouping of the abortion cases.

Bacillus pyogenes was found in one out of fifty-two fetuses by Zwick and Zeller. Holth recovered this organism in one out of twenty-four aborted fetuses. Smith found it in two cases of abortion in which the preceding pregnancy was cut short by *Bacterium abortum* infection. Table 1 shows that *Bacillus pyogenes* alone was found in cases 9 and 22. The dam of the former reacted to agglutination test with *Bacterium abortum* antigen. This organism has been associated with suppurative processes in mastitis, pyelonephritis, and very frequently alone or in conjunction with streptococci in chronic metritis. Whether or not *Bacillus pyogenes* is the cause of abortion in even the very few instances mentioned, or simply penetrates the fetus after its death from other causes, cannot be concluded from the evidence at hand. Wall's findings strongly suggest that this organism may produce abortion.

Streptococci and staphylococci have been found by C. M. Carpenter. In the twenty-five aborted fetuses he found the former alone or with other organisms in four of them, and the latter alone or with other organisms in the same number of cases. He reported these organisms in practically the same proportion in fetuses from pregnant uteri. Carpenter, Williams, and Gilman¹¹ have associated the streptococci with various forms of salpingitis, and it has been found by Wall about as frequently as *Bacillus pyogenes* or in conjunction with *Bacillus pyogenes* in various cases of chronic metritis. Regarding the importance of streptococci in infectious abortion, Carpenter states: "It does not seem possible that these organisms can invade the embryonic or fetal tissue without causing damage." The streptococcus has not been observed, however, by other investigators in connection with this disease, and it is possible that he was confronted with an infection peculiar to his locality.

Bacteria of the colon-aerogenes group at one time were looked upon as a probable cause of infectious abortion. They are, however, no longer considered as such. These organisms are encountered principally when the material examined is not in fresh condition or in cases where the lungs of the fetuses show expansion by air. Such has been the experience of Zwick and Zeller and of Carpenter. Our cases 7, 27, 31, 32, 35, and 37 belong to this group.

Moussu claims that a variety of paracolon has been found by him in western France as the cause of abortion. He gives no details. The paracolon organisms are very easily isolated and recognized, and the failure to find reports of such infection is indicative of its rarity.

CONCLUSIONS

1. As far as the microorganismal agent of bovine abortion is concerned, it has been proved that *Bacterium abortum* is the greatest in importance, in that by its multiplication and activities it produces the necessary changes to bring about abortion, and it has been recovered many times more than all the other organisms mentioned in this connection.

2. There is a noteworthy percentage of abortions in which the presence of *Bacterium abortum* has not been established. In some of these no microorganisms or only inconsequential ones were demonstrated. In a small percentage of cases negative for *Bacterium abortum*, organisms have been found that must be taken into consideration; in this group belong the *Vibrio fetus* and possibly *Bacillus pyogenes* and some of the streptococci.

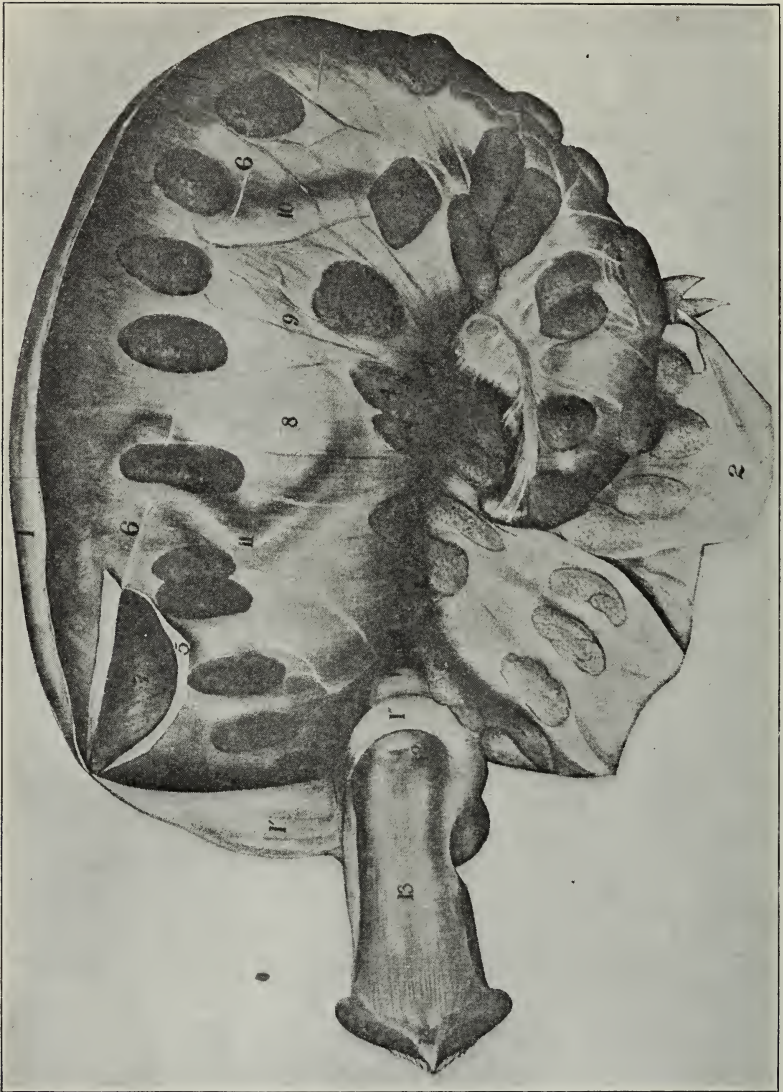


Fig. 3.—Gravid uterus of cow at full term laid open to show fetal sac with faint outline of fetus. (After W. L. Williams.)

1. Dorsal or greater curvature of uterus. 1'. Supra-vaginal pouch of uterus. 1''. Section through uterus at cervix. 2. Right wall of uterine body and horn detached from chorion and turned down. 4. Ventral row of chorionic (fetal) cotyledons. The first two are partly hidden beneath the fetal sac against the cervix. The apex of the fetal sac is rolled outwards showing the median raphe to the left of the last four cotyledons. 5. Dorsal margin of the allantoic sac incised and turned down, exposing 7, the amniotic sac. 6, 6. Dorsal line of allantoic sac, above which only the vascular layer of the allantois extends over the amnion, closely adhering to it. 9. Umbilic cord. 10. Left fetal tibia. 11. Left fetal carpus. 12. Os uteri externum and cervix, showing the cervical canal directed along the uterine floor. 13. Vagina. 14. Vulva.

III

MANIFESTATIONS OF ABORTION DISEASE
WITHOUT DEMONSTRABLE ETIOLOGICAL
FACTOR

BY JACOB TRAUM and GEORGE H. HART

From the information given in the foregoing article, it should be evident that a very high percentage of abortion disease manifestations of cattle is associated with infection by the *Bacterium abortum* of Bang. It has also been established that a certain percentage of abortions is due to other causes. In some instances abortions have been observed where no definite cause could be found but from which the *Bacterium abortum* and other infectious agents have been definitely eliminated. Such a condition has existed in the University dairy at Berkeley and a report of our work on these animals is given in the following pages.

The University has maintained on the Berkeley campus for a number of years a small certified dairy. The herd has consisted of from 24 to 26 cows in the milking barn, 5 to 8 dry cows, and 1 bull. In addition, 6 to 8 heifers are raised each year, making a total varying between 45 and 60 head of animals on the premises. The present bull is a purebred Ayrshire, and was added to the herd in August, 1917. He was raised by the breeder from whom he was purchased, was one year old on September 6, 1917, and probably never was in service until he came to this dairy.

While an effort has been made to keep the dairy supplied with milk cows by raising heifers, it has been necessary to purchase cows from time to time in order to keep the milk supply at the desired level. In 1917, for example, prior to the purchase of the present bull, the previous animal had failed to get the cows properly bred. A number were therefore sold to the butcher and ten outside animals purchased. The trouble at this time was probably due to lack of exercise on the part of the bull. He would not serve cows in estrum that were turned in with him. On being shipped to the University Farm at Davis, where proper exercise could be given him, he again became a satisfactory breeder. In 1918 no additions were made to the herd. In 1919 one first calf heifer was added. In 1920 four animals were purchased. In 1921 to October no additions were made.

In all there were 15 cows and one bull added during the period covered by this report, 11 of which are still in the herd. None of the five cows which were sold for slaughter was disposed of on account of breeding difficulties. Two of them reacted to the tuberculin test, two had mammitis, and one was too low in milk production to be kept in the dairy.

In the purchase of cows for this dairy care is used to prevent the introduction of tuberculosis. The animals are therefore usually purchased from small herds close by or from herds with whose history we are acquainted. Even with these precautions we were not entirely successful in keeping out tuberculosis as attested by the fact that two of the animals later reacted to the tuberculin test. This is the reason for keeping the herd supplied with young stock raised on the premises.

During the years 1917, 1918, and 1919 breeding difficulties of more or less magnitude were encountered, such as an occasional abortion, retained afterbirth, cystic ovaries, and delayed estrual periods requiring expression of the corpus luteum. Agglutination tests with *Bacterium abortum* as an antigen made from time to time on individual animals resulted negatively. Fleischner and Meyer in 1916 made examinations of the certified milk sold in the bay cities for the presence of tubercle bacilli. This resulted negatively, but they did find *Bacterium abortum* a more or less commonly present organism. From the University Certified Dairy it was found in two of three lots of milk collected January 8, 18, and 22, 1917, respectively. Four guinea-pigs were inoculated with each lot of milk. Two of the four inoculated with the first lot and one of the four inoculated with the second lot showed *Bacterium abortum* infection. The remaining nine experimental animals were negative.

All the cows of milking age in January, 1917, except Nos. 1864 and 1869 in Table 1, had been disposed of by August, 1920, and it was decided at this time to make a systematic search for the presence of this organism in the dairy animals.

On August 25, 1920, blood was drawn from the 24 cows in milk, and on August 30 from the remaining 24 head of dry cows, young stock, and bull, a total of 48 head in the dairy at that time. These samples were tested in four dilutions against three strains of *Bacterium abortum* antigen. The results of this test are given in Table 1. It will be observed that all the samples gave a negative reaction to this test, except the blood from No. 2057. The serum of this animal gave a ++ reaction in 0.04 c.c., and a -+ reaction in 0.02 c.c., against all three antigens.

TABLE 1.—AGGLUTINATION TESTS OF BLOOD SAMPLES FROM ALL THE ANIMALS IN THE DAIRY HERD, AUGUST, 1920

EAR TAG No.	ANTIGEN 1 PORCINE				ANTIGEN 80 BOVINE				ANTIGEN 104 BOVINE			
	0.04	0.02	0.01	0.005	0.04	0.02	0.01	0.005	0.04	0.02	0.01	0.005
1964.....	-	-	-	-	-	-	-	-	-	-	-	-
1966.....	-	-	-	-	-	-	-	-	-	-	-	-
1958.....	-	-	-	-	-	-	-	-	-	-	-	-
2030.....	-	-	-	-	-	-	-	-	-	-	-	-
1235.....	-	-	-	-	-	-	-	-	-	-	-	-
1965.....	-	-	-	-	-	-	-	-	-	-	-	-
1223.....	-+	-	-	-	-+	-	-	-	-+	-	-	-
2177.....	-	-	-	-	-	-	-	-	-	-	-	-
1662.....	-	-	-	-	-	-	-	-	-	-	-	-
1441.....	-+	-	-	-	-+	-	-	-	-+	-	-	-
2062.....	-	-	-	-	-	-	-	-	-	-	-	-
1953.....	-	-	-	-	-	-	-	-	-	-	-	-
2026.....	-	-	-	-	-	-	-	-	-	-	-	-
2061.....	-+	-	-	-	-+	-	-	-	-	-	-	-
1967.....	-	-	-	-	-	-	-	-	-	-	-	-
2029.....	-	-	-	-	-	-	-	-	-	-	-	-
1801.....	-	-	-	-	-	-	-	-	-	-	-	-
2055.....	-	-	-	-	-	-	-	-	-	-	-	-
1442.....	-	-	-	-	-	-	-	-	-	-	-	-
2175.....	-+	-	-	-	-+	-	-	-	-	-	-	-
1960.....	-	-	-	-	-	-	-	-	-	-	-	-
1957.....	-+	-	-	-	-+	-	-	-	-+	-	-	-
1864.....	-	-	-	-	-	-	-	-	-	-	-	-
1663.....	-	-	-	-	-	-	-	-	-	-	-	-
2155.....	-	-	-	-	-	-	-	-	-	-	-	-
2171.....	-	-	-	-	-	-	-	-	-	-	-	-
1968.....	-	-	-	-	-	-	-	-	-	-	-	-
1869.....	-	-	-	-	-	-	-	-	-	-	-	-
2170.....	-	-	-	-	-	-	-	-	-	-	-	-
2057.....	++	-+	-	-	++	-+	-	-	++	-+	-	-
1438.....	-+	-	-	-	-+	-	-	-	-+	-	-	-
1445.....	-	-	-	-	-	-	-	-	-	-	-	-
2143.....	-	-	-	-	-	-	-	-	-	-	-	-
2142.....	-	-	-	-	-	-	-	-	-	-	-	-
2027.....	-	-	-	-	-	-	-	-	-	-	-	-
2181.....	-	-	-	-	-	-	-	-	-	-	-	-
1969 (Bull).....	-	-	-	-	-	-	-	-	-	-	-	-
2178.....	-	-	-	-	-	-	-	-	-	-	-	-
2179.....	-	-	-	-	-	-	-	-	-	-	-	-
2180.....	-	-	-	-	-	-	-	-	-	-	-	-
1435.....	+ -	-	-	-	+	-	-	-	+ -	-	-	-
2060.....	+ -	-	-	-	+ -	-	-	-	+ -	-	-	-
2063.....	-	-	-	-	-	-	-	-	-	-	-	-
2144.....	-	-	-	-	-	-	-	-	-	-	-	-
2173.....	+ -	-	-	-	+ -	-	-	-	+ -	-	-	-
2154.....	-	-	-	-	-	-	-	-	-	-	-	-
2183.....	-	-	-	-	-	-	-	-	-	-	-	-
2182.....	-	-	-	-	-	-	-	-	-	-	-	-

Explanation of symbols in table:

- Indicates no evidence of reaction.
- + Indicates slight sedimentation but supernatant fluid turbid.
- + - Indicates more sedimentation than -+ but still a faint cloudiness in supernatant fluid.
- ++ Indicates that after overnight incubation complete agglutination is present.
- + Indicates that after overnight incubation there was not complete agglutination, but on standing for 24 hours longer the tube cleared up.

Animal No. 2057 was an unbred heifer. She was born on the dairy in November, 1917, her dam being cow No. 2029, purchased when middle-aged, just prior to the birth of this calf in November, 1917. The dam is still in the dairy and has had the following breeding record since purchase:

Gave birth to calf 2057 shortly after arriving at the dairy, November, 1917.

Bred March 20, 1918, and calved normally December 28, 1918.

Bred March 20, April 24 and May 25, 1919. Pregnant to last service and calved normally March 15, 1920.

Bred April 20 and May 10, 1920. Pregnant to last service and calved normally February 21, 1921.

Bred June 2, 1921. Pregnant to this service and calved normally March 7, 1922.

Bred May 20, 1922 and pregnant to this service.

Heifer No. 2057 later aborted and will be discussed as Case 2.

CASE 1

Heifer 2154 was the first animal to abort after the general examination of the blood of all the animals given in Table 1. She was born February 15, 1919, and her dam was Cow 1438, which animal has aborted twice and will be discussed as Case 5. At the time blood was drawn this animal was not thought to be pregnant, as she had never been bred by the herd bull. During the spring of 1920, however, there was a history of a stray bull having gotten into the pasture for a few days where this heifer and some other animals were being kept.

On the morning of September 16, 1920, a rider for the water company, whose watershed adjoins our range, saw a heifer in the pasture that had given birth to a premature dead calf, but did not report this until evening. The fetus was seen and covered up with loose dirt by the rider. The forenoon of this same day the dairy foreman noticed Heifer 2154 at the watering trough with tail held out and vulva congested, but did not suspect abortion on account of having no breeding record on the animal. The following day, the water company employee not being available, a search was made of the pasture, but no fetus was found. Coyotes and other predatory animals have been trapped and seen in this pasture and their presence may explain the failure to find the fetus. The animals in the pasture were brought to the barn and upon examination all that were known to be pregnant were still so. On examining No. 2154, although only about 19 months old, the hand could be readily passed into the vagina. The vaginal walls showed some congested areas but the cervix was closed so that one finger could not be inserted into the cervical canal.

The vagina was douched with physiological salt solution, after which the uterus was examined per rectum and found to be enlarged. By massaging it through the rectum with the other hand in the vagina cupped over the cervix, a small amount of mucus containing flakes of yellowish pus-like material was obtained.

This was placed in a sterile bottle and taken to the laboratory. Two guinea-pigs, Nos. 1731 and 1732, were inoculated intra-abdominally September 18, 1920, with the uterine discharge from this heifer. These experimental animals were chloroformed February 7, 1921, and found to be in a normal condition.

This heifer was bred again December 17, 1920, February 22, 1921, and March 28, 1921. She became pregnant to the last service and calved normally January 4, 1922.

Agglutination tests made on her blood drawn April 20 and August 20, 1921 and June 12, 1922 resulted negatively.

CASE 2

At the time the blood samples in Table 1 were taken Heifer 2057 was pregnant to a service July 22, 1920. She had previously been bred on at least two occasions, June 10, and August 10, 1919. From the later date to July 22, 1920, she had been on a hill pasture not exposed to a bull.

On March 27, 1921, while in the pasture, this animal aborted a male fetus due in May from the July service noted above, and the placenta was retained. The animal was brought to the barn and isolated. On March 29, 1921, the membranes were removed manually with little difficulty, the uterus was douched and several ounces of petroleum oil placed in it, following which no further treatment was found necessary.

The fetus was found in the pasture at 4 p.m., March 27, with a few blow-fly larvae deposited about the nostrils, and was brought to the laboratory at 6:15 p.m. On autopsy it showed sero-sanguineous fluid infiltration of the subcutis especially in the axillary and inguinal regions. The thoracic and abdominal cavities were filled with blood-stained fluid. Some gelatinous masses were present around the heart. The spleen measured 20 by 5 by 2 centimeters and showed several subcapsular hemorrhagic blotches. The stomach contents were a stringy turbid mass intermixed with gray flakes and blood. The intestines were apparently normal.

Cultures were inoculated the same evening from the tissues and fluids as follows:

Stomach

1. Shake 2 per cent glycerin, 1 per cent glucose-agar bouillon. Discarded May 17, 1921. No growth.
2. Shake 2 per cent glycerin, 1 per cent glucose-agar bouillon plus serum. No change until April 12, 1921, when a cloudiness was observed on the surface. Subculture and microscopic examination failed to reveal any organism; probably a precipitation of the serum.
3. Cooked blood agar slant. Discarded May 17, 1921. No growth.
4. Cooked blood agar slant. Discarded May 17, 1921. No growth.
5. Cooked blood agar slant in CO₂ chamber. Discarded May 17, 1921. No growth.
6. Cooked blood agar slant in CO₂ chamber. Discarded May 17, 1921. No growth.
7. Cooked blood agar plate in CO₂. Seven colonies; 5 varieties developed. April 2, 1921. All discarded April 12, 1921.
8. Cooked blood agar plate in CO₂ chamber. Many contaminations. May 4, 1921. Discarded April 12, 1921.

Liver

1. Shake 2 per cent glycerin, 1 per cent glucose-agar bouillon. Discarded May 12, 1921. No growth.
2. Shake 2 per cent glycerin, 1 per cent glucose-agar bouillon plus serum. Cloudiness developed as in same culture from the stomach. Subcultured as above. No growth.
3. Cooked blood agar. Discarded May 17, 1921. No growth.
4. Cooked blood agar slant in CO₂ chamber. Discarded May 17, 1921. No growth.
5. Cooked blood agar plate in CO₂ chamber. Overgrown with contaminating colonies and discarded, April 12, 1921.

Spleen

1. Shake 2 per cent glycerin, 1 per cent glucose-agar bouillon. Discarded May 17, 1921. No growth.
2. Shake 2 per cent glycerin, 1 per cent glucose-agar bouillon plus serum. Discarded May 17, 1921. No growth.
3. Cooked blood agar slant. Discarded May 17, 1921. No growth.
4. Cooked blood agar slant in CO₂ chamber. Discarded May 17, 1921. No growth.

Intestines (Colon)

1. Shake 2 per cent glycerin, 1 per cent glucose-agar bouillon. Discarded May 17, 1921. No growth.
2. Shake 2 per cent glycerin, 1 per cent glucose-agar bouillon. Discarded May 17, 1921. No growth.
3. Cooked blood agar, CO₂ chamber. Discarded May 17, 1921. No growth.
4. Two per cent glycerin, 1 per cent glucose-agar slant. Discarded May 17, 1921. No growth.
- 5 and 6. Two per cent glycerin, 1 per cent glucose-agar slant and liver agar in CO₂ chamber. Discarded May 17, 1921. No growth.

All the above-mentioned cultures except those placed in the CO₂ chamber were sealed with sealing wax, as suggested by Theobald Smith. Smears were made from the stomach, liver, spleen, and

intestinal contents and examined in stained and unstained preparations, but no vibrios or other definite microorganisms could be seen.

Five guinea-pigs (three male and two female) were inoculated March 29, 1921, with the tissues of this abort, and one male guinea-pig with colostrum from the udder of the dam, as follows:

Guinea-pigs 1778 and 1779 intraabdominally with intestinal content.

Guinea-pigs 1780 and 1781 intraabdominally with stomach content.

Guinea-pig 1782 intraabdominally with liver and spleen emulsion.

Guinea-pig 1783 intraabdominally with colostrum from dam.

These animals were bled April 11, 1921, and the blood gave a negative agglutination test to *Bacterium abortum* of both bovine and porcine origin. Guinea-pig 1780 was in a moribund condition April 19, 1921, and was bled and killed. The blood gave a negative agglutination test. Post-mortem examination showed many small grayish specks on liver. Lungs contained several small solidified areas. Cultures from liver, spleen, and lungs, kept under observation until July 18, 1921, showed no growth excepting that one shake culture developed a surface growth of a Gram-positive coccus.

The blood of all guinea-pigs used in this work, except Nos. 1731 and 1732 in Case 1 and Nos. 1829 and 1830 in Case 4, was tested for the presence of *Bacterium abortum* agglutinins before inoculation and found negative. This bleeding was done from the ear vein, after the method of Seddon⁶⁹, one-half mil of blood being collected in 4½ mils of carbolized sodium citrate saline solution.

The remaining five guinea-pigs were bled and killed July 11, 1921. Their blood gave a negative agglutination test.

Post-mortem notes on these animals were as follows:

Nos. 1778, 1779, 1781, and 1782, no lesions found.

No. 1783, no lesions found; carcass emaciated.

April 27, 1921, the genital tract of this cow was examined and found to be normal. Agglutination tests, as shown in table 2, were made on blood drawn from this animal, in addition to those given in table 1.

A sample of milk was taken from her May 6, 1921, and inoculated into guinea-pigs. This sample consisted of first milk and strippings in approximately equal amounts, total about one quart of the evening milking. Six hundred mils of this were centrifuged and the sediment inoculated intra-abdominally into guinea-pigs 1795 and 1796 on May 8, 1921. These guinea-pigs were bled July 14, 1921, and again at the time of slaughter, August 30, 1921; the blood gave a negative agglutination test to *Bacterium abortum* of both porcine and bovine origin.

Post-mortem examinations were negative except for some adhesions from peritonitis in guinea-pig 1795. Cultures made from the spleens of the animals on blood-agar remained sterile.

TABLE 2.—ABORTION AGGLUTINATION TESTS, COW 2057

ANTIGEN	MAR. 27, 1921	APR. 18, 1921	APR. 28, 1921	MAY 23, 1921
No. 1 (Porcine)				
0.04.....	++	++	+-	+-
0.02.....	++	+-	-	-
0.01.....	-	-	-	-
0.005.....	-			
B-104 (Bovine)				
0.04.....	++			
0.02.....	++			
0.01.....	-			
0.005.....	-			
No. 3 (Porcine)				
0.04.....	+			
0.02.....	-			
0.01.....	-			
0.005.....	-			
No. 2 (Porcine)				
0.04.....	++	++		
0.02.....	+-	+-		
0.01.....	-	-		
0.005.....	-	-		
B. A. (Bovine)				
0.04.....	++	++		
0.02.....	+-	+-		
0.01.....	-	-		
0.005.....	-	-		
No. 4 (Bovine)				
0.04.....	++			
0.02.....	++			
0.01.....	-			
0.005.....	-			
No. 80 (Bovine)				
0.04.....				+
0.02.....				-
0.01.....				-
0.005.....				

This cow was sold to the butcher and slaughtered on May 26, 1921, because she was the only animal in the herd giving any semblance of what might be termed an agglutination reaction to *Bacterium abortum* antigen. Milk was again taken from her just prior to slaughter and inoculated into guinea-pigs 1817 and 1818 in the same manner as the sample taken May 6, 1921, with negative results.

Two guinea pigs, Nos. 1819 and 1820, were inoculated intraabdominally May 28, 1921, with scrapings from her uterine mucous membrane and supramammary lymph glands. These guinea pigs were bled June 25, 1921, and again on July 14, 1921, and the blood was tested against *Bacterium abortum* antigens of bovine and porcine origin, with negative results. They were bled and killed August 30, 1921. Post-mortem examinations showed them to be normal. Agglutination tests made on their blood were negative. Cultures made on blood-agar from their spleens remained sterile.

CASE 3

Cow 2301 was raised at the dairy and had calved normally April 18, 1918, June 15, 1919, and April 30, 1920.

Following the last parturition she was bred June 28, 1920, and became pregnant to the service. She was therefore two months pregnant when the blood samples in Table 1 were taken. On March 30, 1921, this cow delivered dead twins conceived 275 days previously on June 28, 1920. This pregnancy, therefore, terminated a few days prematurely, which may be accounted for by the fact that there were twins.

The fetuses were male and female and the tissues and organs appeared normal. The membranes were normal except for one small necrotic area present in one fetal cotyledon.

The fetuses were taken to the laboratory for further examination. From the stomach and lungs, respectively, of each fetus the following cultures were made:

Cooked blood-agar	} Sealed and incubated
2 per cent glycerin, 1 per cent glucose shake agar	
Gentian violet fetus medium	
Cooked blood agar	} Placed in 10 per cent CO ₂ chamber and incubated
2 per cent glycerin, 1 per cent glucose agar slant	
Gentian violet fetus medium	
Cooked blood-agar plate	

Smears were made from the stomach and lungs of both the fetuses and examined in stained and unstained preparation. No definite organisms could be found in them.

All the cultures from the male fetus remained sterile, as well as those from the stomach of the female fetus. The cooked blood-agar plate and the fetus medium from the lung of the female fetus under CO₂ showed a few colonies of contaminating organisms (*Bacillus subtilis*) of no significance. All the cultures were discarded May 17, 1921.

On March 31, 1921, guinea pigs were inoculated intraabdominally with tissues of the fetuses as follows:

Guinea pig 1784. Placenta of both fetuses, including a portion of the necrotic area mentioned above.

Guinea pig 1785. Stomach contents of both fetuses.

Guinea pig 1786. Lungs of both fetuses.

Guinea pigs 1784 and 1786 were bled and killed July 2, 1921. Post-mortem examination of No. 1784 showed an abscess on the greater curvature of the stomach from which a streptococcus was isolated. Guinea pig 1785 was bled and killed July 13, 1921. Post-mortem examinations of this animal and of No. 1786 were negative. Agglutination tests made on the blood of all three animals were negative.

EXAMINATIONS OF MILK SAMPLES FROM THE HERD

On May 25, 1921, milk samples were taken from all the cows in the dairy giving milk at the time and composite samples were injected into guinea pigs. The samples were collected as shown below, and two guinea pigs were inoculated with each sample, making a total of twenty experimental animals.

MILK SAMPLES FROM U. C. DAIRY ANIMALS

(Taken from evening milking May 25, 1921. At least 600 c.c. samples centrifuged May 26 and sediment inoculated intraabdominally into guinea pigs May 27, 1921.)

Sample 1. Composite of first milk and strippings in approximately equal amounts total about one quart from cows 2027, 2178, 2299, 1869 and 1662, inoculated into guinea pigs 1797 and 1798.

Sample 2. Ditto from cows 1968, 2055, 2063, 1960 and 1967, inoculated into guinea pigs 1799 and 1800.

Sample 3. Ditto from cows 2029, 2301, 1864, 1435 and 1965, inoculated into guinea pigs 1801 and 1802.

Sample 4. Ditto from cows 1438, 2298, 2300, 2030 and 2026, inoculated into guinea pigs 1803 and 1804.

Sample 5. Ditto from cows 1223, 2060, 2296, 2177 and 2061, inoculated into guinea pigs 1807 and 1808.

Sample A. From vat after milking of cows 2027, 2178, 2299, 1869, 1662, 1868 and 2063, inoculated intraabdominally guinea pigs 1809 and 1810.

Sample B. From vat after milking of cows 2055, 1967, 2029, 2301, 1864, 1960 and 1435, inoculated intraabdominally guinea pigs 1811 and 1812.

Sample C. From vat after milking of cows 1438, 1965, 2298, 2300, 2030, 2026 and 1223, inoculated intraabdominally guinea pigs 1813 and 1814.

Sample D. From vat after milking of cows 2296, 2060, 2177, 2061, 2142, 1957 and 1953, inoculated intraabdominally guinea pigs 1815 and 1816.

Six of these guinea pigs, Nos. 1799, 1801, 1802, 1805, 1810, and 1816, died of intercurrent disease. Blood was obtained from No. 1805 June 16, from No. 1802 June 23, and from No. 1801 June 25, all of which gave negative agglutination tests. The remaining 14 were bled June 25, July 14, and before slaughter August 27 and 30, respectively. Agglutination tests made on these blood samples against *Bacterium abortum* antigens of bovine and porcine origin all resulted negatively.

Post-mortem notes on these animals were as follows:

POSTMORTEM NOTES ON INOCULATED GUINEA PIGS

Guinea pigs 1797, 1798, 1800, 1803, 1804, 1806, 1807, 1808 and 1809. Condition good; no lesions. Cultured spleen on cooked blood-agar.

Guinea pig 1811. Condition good; slight adhesions between left lung and pleura. Cultured spleen on cooked blood-agar.

Guinea pig 1812. Condition good; no lesions. Cultured spleen on cooked blood-agar.

Guinea pig 1813. Condition good; left lung showed adhesions and an abscess. Cultured spleen and lung on cooked blood-agar.

Guinea pigs 1814 and 1815. Condition good; no lesions. Cultured spleen on cooked blood-agar.

All the cultures made from the spleens and incubated under 10 per cent CO₂ pressure remained sterile and were discarded September 23, 1921. The culture made from the abscess in the lung of guinea-pig 1813 showed a growth after two days of a bipolar organism not considered significant.

CASE 4

Cow 1662 was raised at the dairy. First calf born normally August 16, 1918. Second calf born normally September 23, 1919. Third calf born July 13, 1920. At this parturition the afterbirth was retained and had to be treated and manually removed. The uterus was examined August 28, 1920 and found to be apparently normal. This cow was bred October 20 and November 10, 1920, and March 1, 1921, becoming pregnant to the last service. Her blood was examined as shown in Table 1 and again April 26, 1921, and gave negative agglutination tests. The animal was examined and found pregnant July 9. In this examination one hand was placed in the rectum and the other in the vagina. The hand in the vagina clasped the cervix, fixed it and exerted some traction posteriorly.

On the morning of July 29, five months after conception and twenty days following examination, this cow, when brought into the

barn, had fetal membranes protruding from the vagina, but a search of the corral failed to reveal the expelled fetus, probably for the same reason as that mentioned in Case 1. At 4 p.m. that day the cow was examined and the fetal membranes removed. The fetal cotyledons were very yellow in appearance. The chorion had a light pink color, but on close examination this color was found to be streaked with yellow. In three areas about four inches in diameter the capillary congestion was very marked, being unevenly distributed so that the membrane appeared mottled with reddish spots of about the size of a pea. The membranes appeared perfectly fresh with no odor or evidence of decomposition.

Two guinea pigs, Nos. 1829 and 1830, were inoculated with 1 c.c. each of an emulsion obtained by grinding small pieces of tissue from the cotyledons and membranes with the gelatinous material in the membranes and a small amount of sterile physiological salt solution.

Blood was taken from these guinea-pigs by bleeding from the ear August 20, 1921, and the agglutination test with *Bacterium abortum* antigens of bovine and porcine origin was applied to it, with negative results.

The guinea pigs were bled again and killed November 10, 1921. Both were normal on post-mortem examination, although No. 1830 was in poor condition. Agglutination tests made on the blood were negative. Cultures were made on blood-agar plates from the spleen of No. 1829 and from the spleen and testicle of No. 1830 and incubated under 10 per cent CO₂ pressure in a glass jar.

The blood-agar plates were examined at the end of four days' incubation in the CO₂ chamber and showed only a few contaminating colonies. The plate from the testicle of No. 1830 showed only one colony and it was not on the streaked area. These plates were kept in the incubator not under CO₂ for three days longer and were discarded November 22, 1921, having shown no significant organisms.

CASE 5

Cow 1438, the dam of No. 2154, Case 1, was born in the dairy in 1914. Her first calf was born normally in January or February, 1917. Second calf born normally January 28, 1918. Third calf born normally February 15, 1919.

Following this parturition she was bred in May or June, 1919, the exact date not being recorded. Dec. 17, 1919, she aborted twins at about 7 months' gestation, and the afterbirths were retained, necessitating treatment and manual removal.

She was bred again on February 14, 1920, and became pregnant to this service. The agglutination test shown in Table 1 on her blood was negative. She calved normally November 10, 1920. This is an unusually good milk cow and she was not bred again until April 4, 1921, to which service she conceived.

A second agglutination test was made on blood taken from the animal April 26, 1921, with negative results. This animal was examined for pregnancy by the bi-manual method, as in Case 4, September 15, 1921, and found to be pregnant.

During the night of September 25, she aborted the fetus conceived April 4 and had retained afterbirth which had to be treated and manually removed September 28, 1921.

The fetus was brought to the laboratory on the morning of September 26, 1921, and cultures were made from the stomach, lung, and thoracic fluid as follows:

- Three glycerin-agar shake cultures.
- Three glycerin-agar slant cultures.
- Two cooked blood-agar slant cultures.
- Three glycerin-agar plate cultures.
- One blood-agar plate.

All the cultures were incubated under 10 per cent CO₂ pressure. Smears and hanging-drop preparations made from the fresh material did not show any definite organisms. The cultures remained sterile with the exception of two large white colonies on one plate culture and one on another, which were clearly of no significance. The cultures were discarded October 12, 1921, by which time molds had developed on the plates, but nothing in the tubes.

Two guinea-pigs, Nos. 1843 and 1844, were inoculated with stomach contents, thoracic fluid and lung of the fetus September 21, 1921. Blood taken from these guinea-pigs November 16, 1921, gave a negative agglutination reaction with *Bacterium abortum*. They were bled and killed December 8, 1921, and found to be in good condition and normal. Their blood failed to react to *Bacterium abortum* antigen. Cultures were made from the spleens on cooked blood-agar and glycerin-agar and incubated under 10 per cent CO₂ pressure until December 19, 1921, but remained sterile and were discarded.

SUMMARY OF THE CASES

In considering these cases, No. 1 is interesting from the fact that her dam has aborted twice. The possibility should not be overlooked that the bovine uterus in some animals is inherently hypersensitive to overdilatation to the point that expulsive contractions take place at certain stages of gestation, resulting in premature expulsion of the offspring, as has been observed in the human family.

Case 2 attracted attention at the time of the general examination of the blood of all the animals in August, 1920. While her blood serum gave an agglutination reaction to *Bacterium abortum* higher than any of the animals in the herd, it still never reached the point where it could be considered positive. Its agglutinating properties increased somewhat between August 30, 1920, and March 27, 1921, in that the — + reaction in 0.02 dilution of the former date reached a + + in the same dilution on the latter date. Especial significance was given to this on account of the fact that she aborted on the latter date. The history of the animal does not reveal exposure to *Bacterium abortum* infection, and the large amount of work done on her fails to incriminate *Bacterium abortum* or any other infection.

Case 3 is of least significance on account of the fact that there were twins and birth was so nearly at term. Death of both fetuses may have occurred during the parturition, as no attendant was present, and there may have been posterior presentations, or mechanical obstruction of the umbilical vessels may have occurred.

Cases 4 and 5 aborted twenty and ten days, respectively, after a bimanual manipulation of the genital organs in the diagnosis of pregnancy. These two cases, together with other circumstantial evidence, lead us to suggest that such examination may, under certain conditions at present unrecognized, be responsible for abortion. It is the vaginal portion of the examination on which we place the responsibility if any can be placed on this procedure. Its value in the diagnosis of pregnancy in some cattle is so great that it can not well be dispensed with, but if it is a possible cause of abortion, it will have to be done with more care than is at present exercised when the opinion is so generally held that there is no danger of examination causing this phenomenon.*

*In a treatise on sterility of cattle by J. Albrechtsen,¹ of Denmark, translated into German in 1920 and received at this office in July, 1922, the statement is made that even not very thorough or long-continued bimanual examination may produce abortion ("Selbst nicht sehr gründliche und lang dauernde bimanuelle Untersuchungen können Abortus hervorrufen.")

All the cases described occurred between September 16, 1920, and September 25, 1921, a period of about one year. If we exclude Case 3 as doubtful, there still remain four definite cases of abortion in one year among about forty animals of breeding age—10 per cent of premature expulsions of the offspring in this herd without demonstrable cause.

OTHER CASES SHOWING BREEDING DIFFICULTIES

In addition to the cases reported above, Cow 1869 had a retained afterbirth in 1919 and Cows 1960 and 1965 had retained afterbirths in 1920. Cow 1953 had the same trouble in 1921. This last animal was treated in 1919 for cysts in her ovaries and for retained corpus luteum. Following her first calf, June 29, 1918, she did not come in heat until after treatment on March 12, 1919. She was then bred March 15, April 26, and May 28, 1919, becoming pregnant to the last service. She was born in 1914 and considerable difficulty was experienced in getting her pregnant the first time.

Following the conception of May 28, 1919, she calved normally March 12, 1920. She was bred again July 22, 1920, to which service she conceived and delivered twins April 18, 1921. At this parturition one of the fetal membranes was retained and had to be manually removed forty-eight hours afterwards. Little difficulty was experienced in removing the membranes, but considerable discharge from the uterus was observed April 27. She was treated April 29 and 30 with warm uterine injections of physiological salt solution followed by 1 per cent Lugol's solution, after which the discharge ceased.

FEEDING OF THE HERD

The milking cows in this herd are fed dry feed during about nine months of the year, from about June 15 to March 15. During the remaining three months, which constitute our green feed season, they are allowed to run on hillside pasture, getting practically all their feed in this way. The dry feed consists entirely of alfalfa hay as a roughage, with a varying mixture of concentrates. Beet pulp and coconut meal have been regularly furnished. During most of the period covered by this report wheat bran has been used as a third concentrate, but at times this has been replaced with rolled barley. The dry cows and young stock are kept on pasture during all the year, it being sometimes necessary during the winter to feed them a small amount of hay. The bull is kept penned up during most of the time and fed dry feed. During the green-feed season he is usually given a few weeks on pasture.

CONDITION OF ANIMALS TO DATE

On June 12, 1922, blood was again taken from all the animals in the dairy herd, numbering at the time forty-two head. Agglutination tests made on these samples were negative in every instance. From September 25, 1921 to date (August 1, 1922), no abortions have occurred on the dairy. One calf was born dead, but at full term, to cow 1869 on May 25, 1922. Cultures made from this calf remained sterile and two guinea pigs inoculated with the lung and stomach contents, respectively, remained negative and on post-mortem examination August 2, 1922, were normal. There have been four cases of retained afterbirth in this time.

OTHER FACTORS ASSOCIATED WITH BREEDING DIFFICULTIES AS POSSIBLE CONTRIBUTING CAUSES OF ABORTION

A wide variety of causes has been named to account for manifestations of abortion disease, among which may be mentioned certain plants on the range, moldy or otherwise spoiled feed, poisonous substances in the feed, such as ergot, injuries, diseases associated with high body temperature, and the impairment of breeding ability on the part of the bull. In recent years, research has brought out the possibility of, and in a measure definitely demonstrated, positive effects on the reproductive organs of diets restricted to certain plants, also the effects of vitamin and mineral content of feeds upon the reproductive processes. This is the case even though the feeds may contain all the necessary proteins, carbohydrates, and fats to constitute a balanced ration and sufficient in quantity to equal what is known to be required for maintenance and production of normal bodily functions. While some of the above factors have a bearing on the general problem of abortion, there has been a tendency in the past to overestimate the importance of some of them, usually in an effort to prevent the presence of true infectious abortion becoming public knowledge. It is only by keeping in mind all known factors regarding the disease that it is possible to ascertain the truth in regard to individual herds.

Febrile Diseases.—A considerable percentage of a herd of cattle in anthrax-infected territory may develop anthrax with very high fever for several days and be saved from a fatal termination by the liberal administration of anti-anthrax serum. During and immediately following the disease, a certain percentage of the pregnant cows may abort. One would be justified in considering the high fever and

the general disturbances caused by the disease as the direct cause of the abortions, particularly if no abortions had occurred previously in the herd. Abortions have been observed to follow attacks of contagious pleuro-pneumonia and foot-and-mouth disease, which diseases are accompanied by high fever and marked constitutional disturbances. These conditions are comparatively rare, the latter two diseases not existing in this country, and one would not be justified in attempting to explain abortions as the result of slight febrile disturbances accompanying indigestion or other minor conditions.

Injury.—In regard to injury, one case has come under our observation in which the owner of a small herd of cattle milked them in an uneven, wooden-floored stanchion. One cow at times was very nervous during milking, and one evening, on becoming more so than usual, he tied her hind legs with a rope. This made her worse and she finally threw herself in the stanchion. About three days following, this animal aborted a seven-months' fetus. The fetus was examined by us for the presence of *Bacterium abortum* and negative results were obtained in both cultures and guinea pig inoculations. However, stained smears from the stomach showed the presence of vibrios, which organism has been found to be the probable causative factor in abortion in rare cases in cattle and sheep. We did not succeed in getting the vibrios to grow on any of the cultures.

Some weeks later an agglutination test with *Bacterium abortum* antigen was made of the blood of all the animals, nine in number, on this place. A positive reaction was obtained in only one cow, which had been added to the herd since the abortion occurred. This case could therefore have been readily considered an abortion due to injury, but the presence of vibrios tends to throw considerable doubt on this. Abundant evidence is at hand showing cows to be accidentally or intentionally subjected to very extreme physical exertion and rough treatment without any ill effects to the pregnancy then existent.

Poisonous Plants, Spoiled Feed, and Alfalfa.—The statement that plants, such as mistletoe, acorns, and spoiled, moldy, rust-infected feed, or excessive feeding of a single feed, such as alfalfa, are causes of abortion, is based on the most intangible evidence, and if any of them ever cause the premature expulsion of the offspring, it certainly is so rare an occurrence as to be a negligible factor in the extreme prevalence of this disease among our livestock.

The belief that the ingestion of mistletoe by pregnant cattle will produce abortion seems to be more generally held by livestock owners in this state than is the case with any other plant. A supply of it

was therefore collected and fed to two pregnant animals in the University Certified Dairy herd, which is free from infectious abortion. One of these animals, Cow No. 2029, was pregnant three months to a service April 20, 1922, and the other cow, No. 2451, was pregnant five months to a service February 24, 1922. The existence of pregnancy was confirmed in each case by rectal examination. On July 21, 1922, these animals were fed fourteen pounds each of mistletoe and on July 22 each received nineteen pounds additional. Twelve pounds of the material remained and this was fed to cow No. 2451 on July 23, 1922. The short stems with the leaves attached and berries just beginning to form were fed. The cattle ate the plant with apparent relish but no ill effects were produced by it that could be observed. One of the cows had just been dried off, the other was in milk. A small amount of the plant was dropped on the ground from the containers by the cows during feeding and not ingested. This did not exceed a total of two pounds by each animal. No. 2029, therefore, ingested thirty-one pounds and No. 2451 forty-three pounds, which we feel is a greater quantity of this plant than any cow on the range would be able to get in a similar period of time.

In regard to rust, Harms²⁸ recites a case where forty cows were fed for months green feed which was rather thoroughly covered with rust, without any abortions occurring. Albrechts,²² as a result of feeding experiments upon pregnant sheep and goats, came to the conclusion that quantities of smut (*Tilletia caries*), even many times as large as would be consumed in ordinary infected food, did not cause abortion. Pusch,²⁸ in his experiments with smut-infected wheat upon horses, cattle, sheep, goats, and swine, observed no ill effect except an occasional diarrhoea.

A few years ago Haring made a field survey in this state concerning the possible effect of alfalfa feeding on the production of abortion and sterility. Reports on this work covered 1229 cows fed alfalfa alone, among which there were 11.07 per cent abortions and 8.06 per cent sterility; 167 cows fed chiefly on alfalfa, in which there were 23.35 per cent abortions and 11.38 per cent sterility; 355 cows fed partly on alfalfa, in which there were 33.8 per cent abortions and 32.68 per cent sterility; and 452 cows fed no alfalfa at all, in which there were 28.32 per cent abortions and 29.64 per cent sterility. The information on which these figures are based was furnished by the cattle owners and not checked by veterinary examination. They do not indicate that the feeding of alfalfa has any influence on abortion disease.

Popular suspicion of this kind has largely been handed down from the days before bacteriology had made progress in elucidating the nature of infectious diseases, and earlier literature contains many case reports throwing suspicion on these agents as causes of abortion which is not substantiated by any well-planned experiments. Even ergot, the most widely recognized substance as a possible factor in abortion, will not cause this phenomenon unless it has been ingested in quantities sufficient to endanger the life of the animal. It has the effect of stimulating uterine contractions after labor begins but, according to experiments on animals, does not initiate uterine contractions during the gestation period. Thus, Albrecht's investigations on small pregnant ruminants²² demonstrated that twice the therapeutic dose of ergot had no apparent ill effects. Four or five times the therapeutic dose produced a transient effect causing diarrhoea, inappetence, and muscular tremors, but no abortions.

Impaired Breeding Ability on the Part of the Bull.—This is a definite and sometimes serious cause of reduced breeding efficiency. Such a bull may serve cows fairly well or be very slow in service and only a small percentage of the cows bred to him will become pregnant. This may be followed by delayed reappearance of estrum and, after six or eight months, a high percentage of the cows will be found not with calf and low in milk production. Such a condition occurred at the University Certified Dairy with the herd bull in 1917 and with the present bull in the winter of 1920–21. In both cases it was due to improper handling of the animal and lack of sufficient exercise. Over a period of about six or eight months both bulls became very inefficient breeders, but both returned to a normal condition after being given proper exercise and care. In the meantime a considerable percentage of the cows became low in milk production or dry without being pregnant. The problem of properly exercising the bull on the campus is difficult because so many pedestrians walk through the pastures and become frightened at sight of the animal, so that he must be kept in a small corral near the dairy.

The Effect of Diet on Reproduction.—In this field some definite scientific facts have been elucidated in recent years. The rôle of the secondary dietary essentials, commonly termed vitamins, and the importance of a sufficient supply of inorganic substances in the diet, particularly calcium and phosphorus, on the reproductive processes have been but recently recognized. The correlation of the knowledge at hand and additional research will be necessary before their relation to reproduction is fully understood and practical procedures

deduced from them. Nevertheless, the work of Hart, McCollum, Steenbock, and Humphrey^{29, 30} has definitely shown that certain rations, particularly a diet restricted to the oat and the wheat plant, even though balanced, will be inadequate for the nutrition of breeding cows, to the point that the offspring will be born prematurely and either dead or in a very weakened condition.

Evans,¹⁹ in his studies on the estrous cycle of white rats, has been able by restricted diets to keep these animals more or less permanently in estrum and to delay evidence of estrum indefinitely.

Forbes²¹ has shown in his studies on the mineral metabolism of the milk cow that heavily producing animals during lactation are usually utilizing in their bodies and giving off with the milk more calcium and phosphorus than they are ingesting with their food.

The work of Meigs⁴⁵ indicates that this condition when long continued may result in milk yields greatly below previous production records of the individual animals. He was able under certain conditions to enable such animals to attain previous production records by proper feeding during a reasonably long dry period.

Hess³¹ has demonstrated, in his study of rickets in children in New York City, that the amount of vitamins in cows' milk is greater when these animals are on green feed than when stall-fed on dry material. The reduction on changing to dry feed begins to appear shortly after the green feed is removed, showing that the dairy cow cannot store up these substances in her body, and that they must be regularly supplied with the feed. Milk secretion and reproduction processes are very closely associated with each other.

These various conditions may enhance the virulence of infectious abortion when it gains access to a herd of cattle. At the present time means are at hand to determine the presence or absence of infectious abortion, and, in the vast majority of cases where other causes were suspected, investigation has shown the presence of the infectious causative organism. All cases of abortion should therefore be considered as infectious abortion unless, by means of bacteriological or serological examination, or both, this can be eliminated.

IV

DISEASES OF THE GENITAL TRACT OF CATTLE
FREQUENTLY ASSOCIATED WITH ABORTION

By FRED M. HAYES

The disorders of the reproductive organs of the male and female of the bovine species have become a serious menace to the cattle industry and of increasing importance with the development of pure-bred live stock. The perpetuation of breeds, strains, and characters is obviously dependent upon the mating of individuals possessing healthy genital organs capable of fulfilling their function of reproduction. Unfortunately attainment of this ideal is often difficult because of the existence of a variety of diseased conditions in the essential organs. In the following consideration of some of the more important disorders of the genital tract of cows, the effect of these diseases upon breeding efficiency must be kept in mind.

Most of the diseases of the generative apparatus which prevent or delay conception in breeding animals are undoubtedly due to infectious agents. The preponderance of evidence incriminates *Bacterium abortum* as the most important factor in at least starting these infections. Other organisms may be responsible for part of these affections or be associated with *Bacterium abortum* in the production of them. The belief that most of the diseases mentioned in the following pages are closely related to the specific abortion infection is so common that they are frequently grouped as allied to, or sequelae of the type infectious abortion disease caused by the Bang organism.

RETAINED PLACENTA (*Placentitis*)

Retention of the fetal membranes constitutes one of the common symptoms of uterine infection. That this infection is caused largely by *Bacterium abortum* seems evident from the fact that retained afterbirth is common in herds in which abortion is known to exist, and from the fact that *Bacterium abortum* is responsible for initiating an inflammation of the fetal and maternal cotyledons that is the cause of at least 75 per cent of the abortions in cattle. The specific abortion germ apparently is not isolated with any greater frequency in these cases than are species of streptococci, staphylococci, and organisms of the colon-aerogenes group.

The bovine fetal membranes and manner of attachment to the mother are more complex than in other species of animals. At the point where the fertilized ovum lodges in the uterine horn there is soon established a marked change in the character of the mucosa, especially at certain points. Segmentation of the ovum has already begun and the developing fetal membranes become differentiated with the formation of an outer layer known as the chorion, the essential membrane which joins the fetus to the uterus of the mother. This union is accomplished in bovines by the formation by the chorion of cotyledons that unite with already existing, but greatly enlarged, cotyledons on the uterine mucosa of the mother, the latter known as the maternal cotyledons and the former as the fetal cotyledons. The fetal cotyledons are tufts of capillaries lined with a single layer of epithelial cells. These tufts fit into corresponding crypts of the maternal cotyledons, whose crypts are also lined with a single layer of epithelial cells. These points of union constitute in the bovine the only paths for the transmission of nutrition and waste material.

There is at no time during the normal development of a fetus any real adhesion between the maternal and fetal cotyledons. The crypts and tufts are only contiguous or in intimate contact. At the moment that umbilic vessels are severed at the time the fetus is expelled the fetal coverings become dead tissue. The vessel walls which have been distended by the circulating blood collapse because of the separation of the fetus from its membranes. Under normal conditions the collapse of the capillaries of the tufts of cotyledons is sufficient to bring about separation in a short time at these points of union. The normal involution of the uterus combined with the weight of the fetal membranes soon expels the contents of the uterus.

Under the influence of an inflammation induced by some form of infection there may, however, develop a premature separation of the membranes or a retention of them after the fetus is expelled. Either condition can only come about through a previously existing inflammation of the placenta and the uterine mucosa, particularly of the cotyledons of each.

The pathology of infectious inflammation in the pregnant womb is not different from such an inflammation in other tissues. There is an engorgement of the arterial capillaries, stasis in the venous capillaries, with resulting accumulation of red and white corpuscles, together with a migration of the latter, desquamation of the epithelial cells, and transudation of inflammatory fluid. This material constitutes the exudate which accumulates in the chorionic space in such

quantities that it is frequently seen accompanying or following abortion or retained placenta. Whether the membranes are to be retained in the presence of the inflammation depends upon the stage and progress of the infection in relation to the period of pregnancy. Retention is less likely to take place when abortion occurs in the early stages of pregnancy, because the development in size of the cotyledons in the bovine uterus is gradual, depending upon the normal

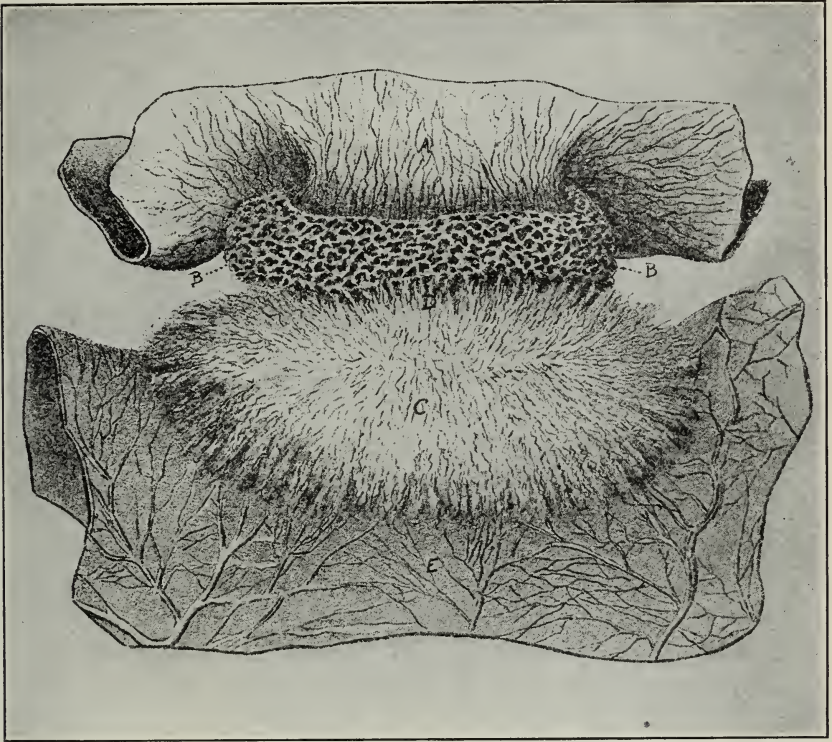


Fig. 4.—A cotyledon from a pregnant uterus. (After special report on Diseases of Cattle, U. S. D. A.) A, Uterine mucosa; B, Maternal cotyledon showing crypts; C, Fetal cotyledon showing tufts; E, Chorion.

growth and enlargement of the fetus and its need for nutrition, and the branches of the tufts of the placenta are not so long and complex, nor the crypts of the maternal cotyledons so deep in the early stages. Later on, however, when the size and complexity of the placental areas grow, the bond of union is firmer and the placenta more likely to be retained even though the calf is born at full time. Retention then depends upon an abnormal union between the maternal and fetal cotyledon. In the cow, according to W. L. Williams,⁹⁰ there are about

one hundred functioning cotyledons in the gravid uterus. Not all of these are commonly affected. The number affected naturally depends upon the progress and severity of the infection. When disease involves only a few of them retention may not take place because the weight of the detached parts may separate those only slightly affected.

The symptoms of retained placenta are usually not difficult of recognition. After the fetus is expelled normally or abnormally, portions of the placenta may be seen protruding from the vulva. This observation is not, however, a safe one upon which to base a diagnosis, because in a uterus that is flaccid and lacking in tone the relaxation of its walls may be great enough to cause a withdrawal of the membranes from the outside to the inside, at least as far as to the cervical canal. A similar condition may develop when the adhesions of the membranes are located in the ovarian end of the uterus only. Obviously the retention of the membranes in such cases as these constitutes a more serious condition since it may not be recognized before more serious complications intervene. As soon as the fetal membranes are severed from the fetus they become a dead mass and undergo putrefaction through the invasion of contaminating organisms entering at the time of birth or already existing in the uterus. In from forty-eight to seventy-two hours after the membranes are retained decomposition may be noted by a fetid odor and perhaps by a vaginal discharge of suppurative material containing shreds of placental tissue. Without treatment the uterus of the rugged cow may continue to expel the decomposing material for a long period of time without apparent serious disturbance to her general condition. In the majority of cases, however, there develops within the generative organs pathological changes that often result in sterility. In the less rugged cow the retention and decomposition of the membranes with the accompanying metritis and absorption of pus soon bring on symptoms of systemic disturbances characterized by lack of appetite, fever, cessation of milk flow, arched back, fixed abdominal muscles, and straining. These symptoms are indicative of the development of a metritis or metro-peritonitis from which latter a large percentage of the cows die even under treatment by a veterinarian. If death does not take place there is strong probability that incurable sterility will develop. Stockmen must recognize that retained afterbirths constitute a very serious menace in that the cow may die or her future breeding efficiency be imperiled.

The treatment of retained placenta is essentially the work of a veterinarian. The condition described above is fraught with so many complications and consequences that only the trained veterinarian is capable of reducing the fatalities and the breeding difficulties to a minimum.

There has, however, been a difference of opinion among veterinarians, who have had wide experience in the treatment of retained placenta as to the details of treatment. Investigation and experience have demonstrated that there is no one line of treatment that will do for every case. The individual cow must be treated in the manner suggested by the extent and progress of the placentitis, and also upon the basis of the resistance of the particular cow in question. The measures applied to the cow with a strong constitution might mean certain death to a less rugged individual.

Over the time and manner of the manual removal of the retained placenta much controversy has arisen. Some attempt the removal of the placenta by forcible traction under any conditions, while others make it a rule not to attempt a removal until at least forty-eight hours after the birth of the calf, provided of course, the uterus is undergoing involution. Here again this part of the treatment depends upon the conditions found in the individual case.

Numerous attempts have been made to cause the afterbirth to be expelled by some artificial means or through the use of drugs administered internally. There is no satisfactory evidence that any drugs will immediately detach the adhering cotyledons. In 1917, R. R. Shaw⁷⁰ described a method, with a report of three cases, of injecting normal salt solution into the ends of ruptured umbilical vessels. This method has never been employed by us, neither have we seen further reports on its use and value. From a study of the pathology of retained placenta, it is apparent that this method of procedure would have limitations as great as any other form of removal.

In the manual extraction of the retained membranes the operator must realize that under the existing inflammation any undue tearing away of the adhering cotyledons may so denude the surface of the uterine mucosa that fresh portals of entry of infection may be opened up, with the consequent danger of absorption of the pus. Unless the membranes can be detached with comparative ease it is much better to allow them to remain and to douche the uterus with some non-irritating sterile fluid to remove accumulated pieces of placenta and pus that may act as food material for the organisms if left in the uterus. Douching, however, is not applicable to every case of retained afterbirth. An accurate diagnosis must be made as

to the size of the cervical opening and the contractile powers of the uterine walls. The douche should be applied, not for the purpose of disinfecting the uterine cavity, because this is practically an impossibility, but for the purpose of washing out the material which might be absorbed or furnish nutrition for the bacteria. Care should be exercised that the return flow of the fluid is unobstructed and that the uterus is able to expel it, or can be so handled through the rectum as to empty it of the fluid injected.

The absence of a rupture must be ascertained before any fluid is introduced into the womb. Fatal consequences from peritonitis usually follow douching of a ruptured uterus.

The saline solution should be used as hot as the hand will stand and allowed to flow in and out of the uterus until the fluid coming out is clear. After the daily application of this form of douche for two or three days the membranes may often be removed with little difficulty. Another very satisfactory method of treating the retained placenta without removing it immediately consists of injecting about one pint of sterile neutral mineral oil into the cavity, once a day for two or three days. The oil is soothing and experience shows that decomposition of the membranes is prevented or considerably delayed by its use. There is apparently little need for the use of boric acid or iodoform in combination with the oil. The protruding membranes should never be allowed to hang any distance from the vulva. The hind parts of the cow, and many times the udder, are contaminated when in contact with the dirty fetal membranes. The exposed membranes also invite the attack of extraneous organisms, which bring about a decomposition that may extend past the cervix and into the womb, and complicate the infection already present. The membranes should be slightly pulled from the vulva and clipped off.

Before the hand is introduced into the vagina and uterus for the purpose of removing an afterbirth or making a diagnosis, it is absolutely essential that sanitary precautions be taken to prevent the introduction of contaminating organisms from the outside. The external parts of the cow around the anus, vulva, buttocks, escutcheon, and tail, should be thoroughly scrubbed with warm water and soap and this operation followed by an application of a disinfecting solution. The hands and arms should likewise be scrubbed with water and soap; the finger nails trimmed and dirt removed from beneath the nails. The arms and hands should be well lubricated with oil and it is well at this stage to introduce about one pint of neutral oil into the uterus with a soft rubber catheter, so that sufficient lubrication will be available to protect the inflamed surfaces of the cervix

and uterus against undue irritation by manipulations with the hand. If the examination discloses the fact that there are numerous cotyledons still adherent it may be advisable to delay the manual removal and introduce more oil, leaving enough of the membranes lying in the cervix to aid in keeping the latter dilated. Attempts must be made to remove the placenta, however, when there is danger of the cervix contracting and imprisoning parts of it. In our experience a condition of this sort is more likely to produce an unfavorable outcome than the forcible tearing away of the adhering placenta, provided the latter is done as carefully as possible.

In cases in which there has been considerable necrosis of the membranes and particularly of the maternal cotyledons, the hot saline douche should be carefully introduced. Highly inflamed uteri that have contained a putrefying mass for several days are easily ruptured by douching and by exploration with the hand. After siphoning off the fluid and drying the cavity of the uterus as nearly as possible, a capsule full of equal parts of iodoform and boric acid may be emptied into the uterine cavity or one pint of neutral oil introduced with a soft rubber catheter. When the membranes have come away under some of the treatments discussed above, the uterus cleansed, and the oil or powders introduced, the treatment for retained placenta *per se* has terminated. This latter statement does not mean, however, that there are not other conditions, which were in the main responsible for the retention, that may need further attention if the animal is to become a regular breeder again.

The earlier expert attention is given to the treatment of retained afterbirth the more probability there is that the life of the cow will be saved, her breeding efficiency remain undiminished, and her milk supply affected to the minimum. It is impossible within the space of this bulletin to go into the detail of the pathology of the many different conditions which the veterinarian recognizes under the general term of retained placenta. If the cattle owner is without competent veterinary service it is much better for him to keep his hands entirely out of the uterine cavity in these cases and confine his treatment to the douching of the vagina with hot saline solutions once daily. One potent cause of sterility in the past and at the present is interference by the livestock owner with retained placenta without the requisite knowledge of the anatomy and pathology of the genital tract of cows and of sanitary procedure. Especially has harm been wrought by the often advocated douching of the uterus with strong disinfectants.

METRITIS AND ENDOMETRITIS

These terms designate an inflammation of the uterus proper. The types of inflammation in this organ and the parts of the organ affected may vary to a considerable extent, probably dependent upon the predominating infective organism. Usually a catarrhal and a suppurative metritis may be distinguished, the later frequently resulting in and constituting a pyometra. In some cases where the mucosa only is involved the term endometritis is applied. It is always difficult, however, to determine clinically the extent of the inflammation and tissues involved. In every case of abortion and retained after-birth some form of metritis exists. It may involve only the endometrium or it may include the muscular walls of the uterus and even the serous surface.

The cause of these conditions has not been satisfactorily worked out from a bacteriological point of view. Numerous types of organisms have been found in diseased uteri but their etiological significance has not been demonstrated beyond that which is credited to the Bang organism. Eggink²⁰ records results of bacteriological examination of twenty cases of endometritis as follows: "*Bacillus tuberculosis* 12 times, *B. pyogenes bovis* 14 times, streptococci 12 times, *B. coli* 6 times, staphylococci 5 times, *B. proteus* 3 times, *B. subtilis* once." C. M. Carpenter¹² found organisms of the streptococci group predominating in lesions in the genital tract of sterile cows, but the character of the lesions is not noted in relation to the organisms isolated. *B. pyogenes* has also been found to be associated with metritis and Beaver⁶ in a report on the bacteriology of sterility in cows, says: "It seems from our studies that streptococci and *B. pyogenes* play the most pathogenic rôles in metritis and other inflammatory conditions of the genital tract of the cow."

The clinical manifestations of inflammation of the uterus depend upon the type and extent of the inflammation. In acute cases of septic metritis, which at times may occur independent of abortion or retained placenta, the endometritis becomes aggravated through a new type of infection or a lowered resistance of the animal. The exudate is usually a dirty grey or reddish-brown color. Pus and parts of necrotic tissues which may include the maternal cotyledons are discharged. There may or may not be a rise of temperature; dullness and prostration are evident. The extremities of the animal are usually cold and there is a disinclination to move on account of the painful uterus. Diarrhoea frequently comes on and consists

of thin, dark, foul-smelling feces. Death may occur within five or six days and if recovery takes place the animal is usually sterile, due to permanent changes having taken place in the generative organs. In the milder types of inflammation which include endometritis there may exist no appreciable disturbance in the general health of the animal. A small amount of greyish-white pus or necrotic tissue débris may be discharged from the vulva for a considerable period of time. The appetite and milk flow are very little disturbed.

Aside from the danger of death occurring from metritis and its complications, the most important outcome is the temporary or permanent sterility supervening. The pathological changes which have been noted in the uterus following acute or long-standing inflammation are well described by Hallman²⁷ as follows: "The lesions observed in varying degrees in the different cases are mucoid degeneration of the superficial epithelium, local and diffuse fibrosis of the uterine mucosa, leucocytic infiltration of the stroma and gland lumina and degeneration and disintegration of the glandular epithelium with diminution in the number of glands. In the majority of cases the anatomical alterations are comparatively few and it is hardly conceivable that failure to breed was the result of loss of functional tissue of the uterine mucosa."

It has not yet been determined to what extent organic changes in the uterus under inflammation are responsible for failure to breed. The probabilities are that the functional disturbance of other necessary parts of the generative apparatus induced by the inflammation in the uterus is as important a factor in sterility as the specific changes in the walls of the uterus. From a study of these conditions it seems impossible to have any acute or long-standing disturbance of the uterus proper without the adjacent cervix, oviducts, and ovaries becoming involved.

The treatment of all forms of metritis must, of course, vary according to the clinical manifestation. The prognosis in acute septic metritis is always grave and the treatment must in the main be symptomatic with such specific measures applied to the uterus as will aid in removing necrotic membranes or purulent material that may aggravate the toxemia through absorption. Comfortable quarters, fresh cool water, laxative food, if the animal will eat, and due attention to the need of stimulants are essentials of the treatment. The attention that may be directed to the uterus itself will depend upon its condition. If the cervix is sufficiently open to allow the passage of a soft rubber catheter, the introduction of hot saline

solution once a day is helpful if there is inflammatory débris that should come away. It must be kept in mind that in the acute septic form of metritis it is dangerous to handle some uteri to any great extent. Their walls are soft and friable, easily mutilated, and their rupture practically always induces a fatal peritonitis.

In the more chronic forms, characterized by a low form of inflammation and exudations, such as is seen in endometritis, the weekly irrigation of the uterus with proper instruments, together with the treatment of the cervicitis which may exist, brings about recovery and restores the breeding of the animal in the majority of cases. W. L. Williams has long advocated the use of $1\frac{1}{2}$ to 2 per cent Lugol's iodine as a douching material for these latter cases. In the light of his own and our experiences his recommendations are amply justified. The solutions to be introduced into the uterus may be carried by the Albrechtsen tubes or some modification of them, or by soft rubber catheters. The cleansing of the uterus of its exudate is facilitated by massage of the uterus through the rectum. In many cases the uterus may be emptied through massage alone. In doing this the hand is introduced into the vagina and the cervix grasped. The other hand is employed in the rectum in massaging the cornua in the direction of the cervix. In certain cases the tone of the cornua is materially benefited by massage through the rectum once or twice a week. Some veterinarians who have had considerable experience with this type of inflammation and sterility do not apply forceps or use tubes in many of the cases, depending entirely upon manual manipulation of the uterus.

Suspensions of dead organisms (bacterins) have been advocated as an adjunct to the above treatment. No authentic reports have been made that these are of any great service in this condition. Theoretically they should be of some benefit in some types of cases. We have not used them.

It is useless to breed a cow with an existing metritis of any type and experience demonstrates that not only will the animal probably fail to conceive or if conception takes place the fertilized ovum soon perishes unobserved, but the existing metritis or cervicitis is also aggravated.

PYOMETRA

The term pyometra is used to define a metritis in which the walls of the uterus are relaxed and flaccid and considerable accumulation of pus has taken place. It is usually a chronic condition where the excessive formation of mucus under inflammation has given away

to a purulent exudate and in long-standing cases to the destruction of the endometrium. This condition is one of the most common forms of disturbance of the uterus that interfere with successful breeding. The disease is recognized by the purulent discharge from the vulva, many times noticeable only when the cow is in a recumbent position. The constant discharge of thick, creamy pus suggests pyometra and also that the cervix is open and flaccid. In other types of this infection diagnosis is possible only upon rectal examination and catheterization of the womb. The cervix in the latter types either has not lost its contractile powers, or it is so diseased and swollen that the exit of the pus is prevented. In these cases two types are again recognized. One which is found upon rectal palpation, in which one or both cornua are felt distended with the accumulated pus. Usually both cornua are equally distended and the walls feel soft and relaxed and without tone. Cases have been observed in which semi-solid masses that later proved to be inspissated pus, could be felt in the cornua. In the other type recognized by catheterization only, a small quantity of pus is imprisoned and careful watch must be kept of the irrigating catheter for the flakes of pus coming out diluted with the irrigating fluid. It has been stated that no case of pyometra can exist without a cervicitis, but that a cervicitis does exist without the pyometra.

The important feature of this condition is the frequency with which the infection extends to the Fallopian tubes and to the cervix, resulting in a salpingitis and cervicitis.

The treatment of pyometra in general is not different from that previously described, employed for a chronic endometritis. The uterus must be evacuated of its contents by massage or by catheterization, or both, and infection destroyed. This latter effect can only be accomplished through irrigation and massage, which act in assisting nature in bringing together its own defensive elements. In some cases of pyometra the corpus luteum is retained. It should be expressed in all cases of pyometra. Extraction of the corpus luteum under these conditions aids materially in bringing about contraction and tone to the walls of the uterus.

In the long-standing cases of pyometra in which rectal palpation discloses thick, fibroid walls of the cornua, the prognosis is unfavorable for recovery of the breeding functions. The probabilities are that the endometrium has been destroyed and fibroid degeneration of the walls with possible sclerosis has taken place.

CERVICITIS

This term defines an inflammation of the cervix. This segment of the genital tract is from three to five inches long, and from two to three inches thick, forming the connecting link between the vagina and the uterus. Its walls are normally very thick, dense, and rigid. The canal is tortuous, because of the presence of circular muscular fibers causing the mucous membrane to be thrown into folds. Under healthy conditions and in the absence of heat, the canal is closed and can be dilated only with difficulty. In estrum and parturition it freely dilates, and in certain diseased conditions remains open and relaxed. Located in the mucosa of the organ are numerous mucous glands, which, under the influence of normal heat and inflammation secrete and discharge mucus. When pregnancy is established a mucous plug or seal is established in the canal through the production of material from these glands. This latter function is perhaps one of the most important to be credited to the cervix. The seal acts as a barrier between the uterus and the vagina in pregnancy, thus preventing to a great extent the passage of organisms from the vagina.

In cervicitis we see inflammations in varying degree of this part of the genital tract. It may at times be so severe that the spermatozoa of the male cannot successfully pass the abnormal secretions encountered in the canal. In other cases the inflammation may be so marked that the swollen and tumefied cervix successfully prevents the entrance of the spermatozoa. Recent investigations have also shown that inflammation of the cervix is intimately related to diseases of the oviducts and ovaries. In fact, there is great physiological and pathological sympathy among the different parts of the generative apparatus.

Cervicitis is not generally recognized until examination is made for the purpose of diagnosing the cause of an existing sterility, or in the treatment of some of the conditions heretofore discussed. It is indicated in its simplest form by swelling and redness of the mucosa of the posterior extremity extending into the vagina. Here the external mucous fold may be seen greatly swollen and the cervical folds partly everted. As before mentioned the canal itself may be found dilated through a relaxation of its muscular fibers to the extent that the tortuous canal is entirely obliterated and two or more fingers may be introduced. At other times the canal is entirely closed and muco-purulent material may be observed on the surface of the folds or seen oozing from out the depths of the canal.

W. L. Williams⁹⁰ states that the greatest danger from cervicitis generally arises after fertilization, when the infection tends to inhibit the prompt physiological sealing of the canal and is free to attack the fertilized ovum immediately on its arrival in the uterine cavity, and that cervicitis is the fundamental lesion in abortion and retained fetal membranes.

The treatment of cervicitis should be directed toward the disinfecting of the cervix. For this purpose Lugol's solution or tincture of iodine is successfully employed. In applying iodine to the cervix, a vaginal speculum may be introduced or the cervix may be grasped with uterine forceps and drawn back to the external vaginal opening. In most cows the external parts of the cervix can be brought into view by the use of one or two forceps. Inasmuch as the vaginal mucosa is more sensitive to disinfectants and iodine than the cervix, care should be taken that none of the full-strength iodine comes in contact with the vagina. This can be prevented by protecting the vaginal part around the cervix with absorbent cotton. After drying the cervix with cotton or dilute alcohol a pledget of cotton supported by forceps is saturated with full-strength iodine and applied liberally over the *os* and including the entire cervical canal. This treatment should not be repeated under ten days because considerable irritation of the cervix is caused by the application of iodine and there is some desquamation of cells. This is necessary, however, for any real disinfection of the cervix to take place and is followed by a prompt attempt at healing. Good results may also be obtained in the milder forms of cervicitis where the inflammation is confined principally to the external *os uteri*, by douching at least twice weekly with hot saline solution. *Mild* disinfectants may be employed in place of the saline.

SALPINGITIS

Salpingitis is a disease of the Fallopian tubes or oviducts. The oviduct is a small tortuous tube extending from the apex of each cornua to the corresponding ovary. At the ovarian end of the tube there is a fimbriated expansion in close contact with the ovary into which the ova are discharged from the surface of the ovary at ovulation and conveyed to the uterus. The mucous surface of the oviduct is lined with ciliated columnar epithelium. The cilia aid in transporting the ovum, fertilized or unfertilized, through the tube into the cornua. The spermatozoa of the male pass up the tube in search of the ovum. Fertilization may take place between these two cells in the fimbriated expansion or somewhere in the oviduct, but implanta-

tion of the fertilized ovum under normal conditions should take place only in the uterus.

Salpingitis has long been considered an important factor in the sterility of women and in late years veterinary investigations have brought to light the fact that disturbances of the oviduct in cattle are very common and are responsible for a considerable portion of sterility encountered in cattle. It has been observed in cattle of all ages, including those that have never given birth to a calf, and in certain herds this particular type of infection seems to be endemic. Concerning this latter observation, Carpenter, Williams, and Gilman¹¹ published the following table which gives in brief the relation of salpingitis to the breeding of a herd which they had under observation:

Total cows in herd	87
Clinical salpingitis	23, or 26.4%

Out of 23 cases of salpingitis:

Sterile	14
Aborted prematurely or retained placenta	6
Reproduced normally	3

The disease appears to be essentially of infectious origin and to be an extension of infection from some other part of the genital tract. Researches indicate that infection of the tube is very likely to develop following a retained afterbirth, metritis, and particularly a cervicitis. The organisms most frequently encountered are *Streptococcus viridans* and staphylococci. These types of organisms have also been isolated from adjacent diseased parts of the genital tract. The significance of the presence of these organisms as a specific cause of salpingitis has not as yet been fully determined.

Most cases of salpingitis are diagnosed upon autopsy, or indirectly when no other cause for sterility in a given case can be determined. The autopsies upon sterile cows in which disease of the tubes has been found have demonstrated two things; one that salpingitis in its different forms is responsible for a far greater number of the cases of sterility than heretofore recognized, and second, that sufficient pathological changes may be present in the oviduct to prevent conception and yet not be clinically evident. Some clinicians have maintained that a diseased condition is indicated whenever the oviduct is easily palpable by rectal examination. This is not entirely true, however, because the normal sized oviduct is palpable, though considerable skill and practice are necessary to feel it. Therefore this

is not a safe criterion to follow in diagnosing disorders of the tube because alterations sufficient to prevent conception may be present in the normal sized tube.

The gross and microscopic changes found in salpingitis of cattle are best described by Carpenter, Williams and Gilman¹¹ in the paper referred to above as follows:

On section a thick, yellowish, viscid exudate, appearing like pus, usually oozes from the cut end of the tube, but on microscopical examination only epithelial cells, a mucoid material, and occasional bacteria are seen. Histologically, the conditions observed in the tube are usually some form of a catarrhal inflammation ranging from an acute exudative endosalpingitis to a chronic catarrhal inflammation of all layers with obstruction of the lumen by granulation tissue in the more chronic cases. In acute exudative endosalpingitis there is merely a fibrinous or serous exudate into the lumen, with hyperemia of the mucosa and loss of cilia in many instances. The chronic form is characterized by frequent loss of the lining epithelium, a profuse exudate in the lumen, thickening of the folds by a productive inflammation, and the resulting narrowing, more or less, of the lumen. In the acute catarrhal form affecting all layers, the muscularis and serosa show hyperemia and more or less exudation between the muscular fibers apart, is seen occasionally, and even when present is supposed to be due to a prolonged effort to expel the constantly produced exudate. The chronic form differs from the acute condition by the fact that all coats are, as a rule, affected. Partial or total atresia of the lumen is often brought about, and frequently the intermuscular connective tissue is increased, causing more or less atrophy of the muscular fibers. A cellular exudate into all coats is usually seen, particularly in the mucosa. As has been mentioned before, in hydrosalpinx there is occlusion of the lumen at one or more points, causing an accumulation of the serous fluid. The mucosa in these cases is not materially altered. The epithelium is usually intact, the wall being much thinner and more or less translucent, due to almost complete atrophy of the muscular and serous coats.

The diagnosis of the pathological conditions above described is naturally very difficult to make. In those cases where there have developed adhesions of the pavilion of the oviduct to the ovary or other gross lesions of the duct or in the peritoneal fold that supports it, the condition may be recognized upon rectal examination.

The specific treatment of any form of salpingitis is not as yet well understood and little hope of successful results can be offered. The best treatment is prevention and the preventive measures are best carried out by early and careful treatment of the other parts of generative system that may be diseased. Prompt attention to retained afterbirth, metritis, etc., constitutes the best prophylactic treatment of salpingitis.

CYSTIC DEGENERATION OF THE OVARIES

The cow seems to be more subject to ovarian trouble than other animals. Cystic degeneration is the most commonly observed ovarian trouble in this animal. In one or both ovaries a diseased condition frequently develops, originating either within the Graafian follicle or corpus luteum, which is characterized by degenerative changes and an excessive accumulation of serous fluid. These cysts often grow to enormous size, causing the ovary to be four or five times its normal size, and destroy a large part of the ovarian tissue (Figs. 5 and 6). The degeneration of the ovaries interferes with ovulation and usually results in a cow becoming a nymphomaniac (chronic buller).

The cause of cystic ovaries is as yet not well understood, although considerable work has been carried out in an effort to find the etiological factors. Observations and studies have indicated that the development of cystic ovaries depends upon a disturbance of the generative system, either through infection or functional disorders related to nutritional deficiencies. An extension of the inflammation and infection which is so frequently found in the Fallopian tubes may be one potent cause for this and other diseases of the ovary. It is very common in such cases to find trouble in the other parts of the organs, particularly in the cervix. We have observed that in the majority of cases of long-standing cervicitis requiring treatment, there is a strong possibility of cystic ovaries following. We, as well as others, have succeeded in relieving this disease of the ovaries by rupture of the cysts and correction of the cervicitis.

As a rule, every cow affected with cystic ovaries is a non-breeder and remains so as long as the degenerative changes exist in the ovaries. The estrous cycle is interfered with and usually the cows are recognized as chronic bullers. The symptoms are very characteristic. After the disease has existed for some time marked changes occur in the form of the animal (figs. 7 and 8). The sacro-sciatic ligaments undergo relaxation which in turn modifies the form of the muscles of the rump. The external gluteal muscles visibly sink. The ischium and sacrum seem to be elevated and the anterior end of the ileum depressed so that the "tail head" appears unusually high. There are depressions on each side of the base of the tail and the vulva appears enlarged and flabby. Also the animal may take on some of the characteristics of the bull, indicated by an enlargement of the muscles of the crest of the neck. Heat seems to be present most of the time and the animals will show it by the usual symptoms in an exaggerated

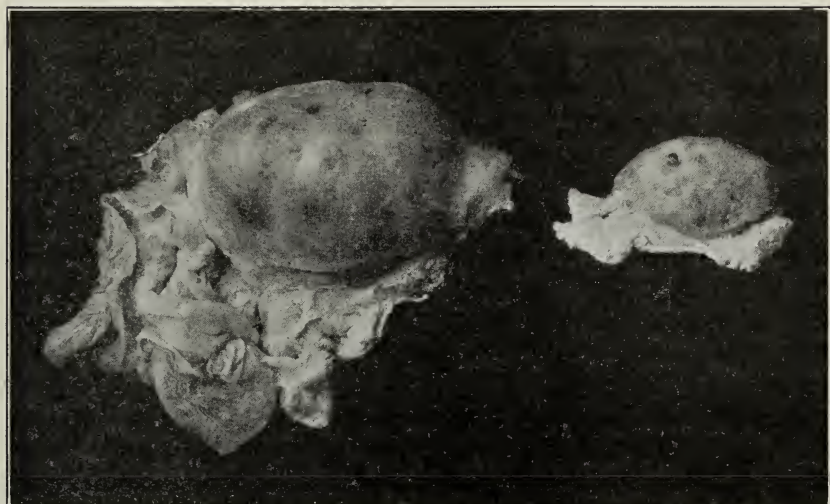


Fig. 5.—Enlarged cystic ovary on left with normal ovary on right. ($\frac{2}{3}$ natural size.)

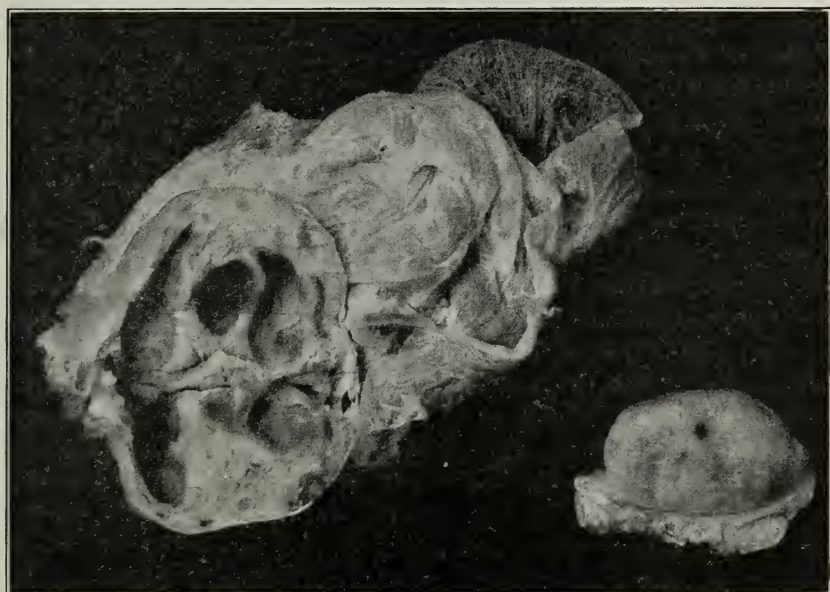


Fig. 6.—Cross-section of the cystic ovary (left) shown in fig. 5. Note the cavities which contained the cystic fluid before sectioning.

form. Rectal palpation confirms a diagnosis of cystic ovaries, suspected from the observation of the above symptoms. Upon grasping the ovaries through the rectum one or both of them may be found to be greatly enlarged, irregular in contour, and one or more cysts of different sizes may protrude from the surface. This disease is particularly important because of the sterility which it produces, the

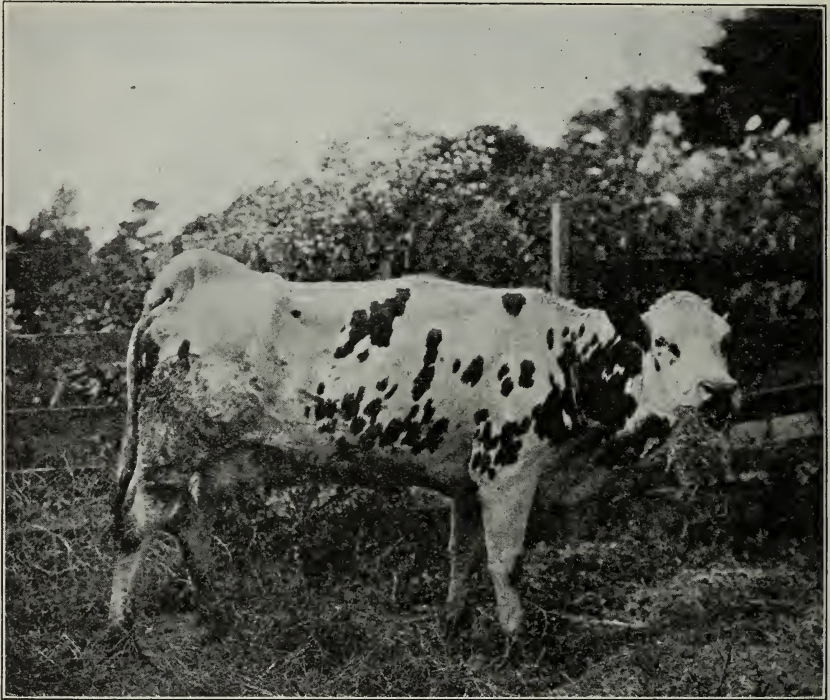


Fig. 7.—Cow with long-standing cystic ovaries. Note the abnormal form of the rump and tail head.

reduced milk flow occurring, and the fact that high producing cows seem to be more commonly affected. In this connection it may be stated that there is growing evidence that cystic ovaries may be related to a mineral food deficiency brought about through the loss of mineral matter in the production of large quantities of milk.

The treatment of cystic ovaries consists of rupture of the cysts through the rectum or the vagina and the simultaneous treatment of any other disordered parts of the generative organs. Albrechtsen² states that nymphomania and cystic ovaries have responded to treatment of the metritis and pyometra which he says always coëxist. Out of 107 cows, 86 per cent were completely cured by him when the

uterus was cleared of its metritis. Our own work confirms the idea that the cervicitis, metritis, etc., must be relieved before we may expect any favorable changes in the ovaries when these are coëxistent with them.

In rupturing cysts of the ovaries the hand is introduced into the rectum and the ovary grasped through the wall of the rectum, pressure being applied upon the protruding cysts. Usually the membrane covering the cyst is so thin that rupture soon occurs. Sometimes, however, it is unyielding and the operation of rupturing must

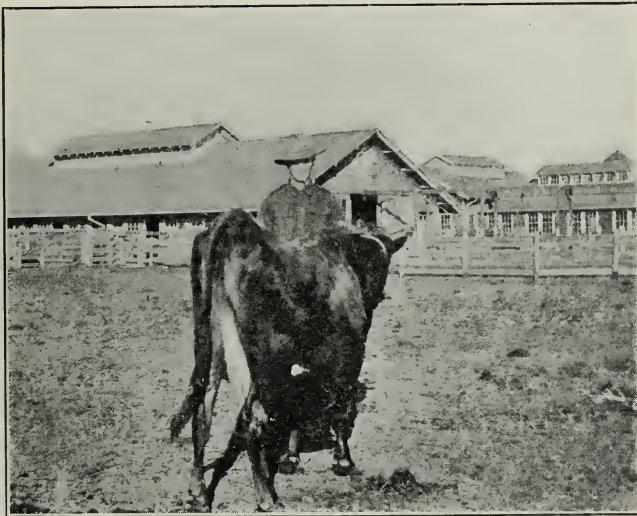


Fig. 8.—Cow with cystic ovaries of two years' standing. Note conformation of rump region.

be carried out through the walls of the vagina with the other hand. This vaginal wall is not so easily lacerated with the fingers as the wall of the rectum.

Cows with cystic ovaries that show symptoms of nymphomania should be kept by themselves and not allowed to copulate with the bull until there is some evidence that the animal has recovered and is capable of conceiving. When a chronic buller is harbored with the rest of the herd in pasture or corral she disturbs the other cows by continually mounting them or allowing the other cows to mount her. Besides the appreciable effect this would have upon the milk flow of the other cows there is danger of fractures and other injuries occurring.

DISCUSSION

In the preceding pages only a few of the more common diseases of the genital organs of the cow have been considered. The space of this bulletin will not permit a fuller discussion of these and many other diseases that interfere with reproduction. In addition to the diseases herein mentioned—and the diseases of the bull have not been considered—there are many others which may be classified under such headings as alterations in development and growth of the organs; congenital defects; specific diseases, such as granular vaginitis, tuberculosis, and actinomycosis; tumors; and functional disturbances brought on by overwork, improper feeding, starvation, mineral deficiencies, and excessive sexual use.

The attention of the breeder is called to the fact that the diseases of generative organs of breeding animals are numerous, widespread, and call for the most expert attention in order to preserve and maintain the breeding efficiency of the herds. And further that there is no one cause why a cow will not breed, nor any one treatment that will meet all cases of sterility.

The most frequent, and also the most difficult question, which the Division of Veterinary Science is called upon to answer in correspondence with breeders is the one concerning the cause for and treatment of certain non-breeding cows in their herds. A study by the stockman of some of these pages will demonstrate how futile it is to attempt to make a diagnosis and recommend treatment without having an opportunity to examine the cows in question, or the conditions under which the animals are maintained. The breeder can reduce the diseases of the generative organs and the resulting sterility to a minimum by the practice of good animal husbandry and by the application of the principles of sexual hygiene and sanitation to the breeding herd.

V

THE DIAGNOSIS OF PREGNANCY IN CATTLE

BY GEORGE H. HART

THE ESTROUS CYCLE

The term estrous cycle is used to indicate the various phases of activity through which the genital organs of the female mammalia pass. These changes recur in more or less periodical manner during the life of the animal, from the time she becomes sexually mature until she passes the age of reproductive activity. In some species there is what is known as a breeding season during which the female shows sexual excitement, and she remains quiescent through the remainder of the year. At this time there may be only one estrous cycle or several cycles may follow each other regularly unless pregnancy intervenes. Animals having only one estrous cycle during the breeding season are termed monestrous, while those having more than one are known as polyestrous. In a few species, the male also has a definite breeding period, as in the deer, and the term "rutting season" is applied to it. In the bovine, as in the majority of species, the male has no definite breeding season and will breed at any time of the year. In some species a single heat period of the female is followed by a long period of rest. The dog furnishes an example of this type of monestrous sexual activity. In the cow, which is a polyestrous animal there is no definite breeding season, and estrous cycles follow one another regularly throughout the year, unless pregnancy intervenes, requiring about twenty-one days for their completion.

In each cycle there are definite stages to the changes which take place. Marshall⁴⁰, who has published an extensive treatise on the physiology of reproduction, has divided the period into four phases to which he has applied the terms used by Heape, of proestrus, estrus, metestrus, and diestrus.

The proestrual period is usually of very short duration in cattle, and in these animals it is not definitely separated from the estrual. Where unbred females, however, particularly heifers, have access to a bull, it is not uncommon to note an animal to apparently be attracted by the bull and remain in close association with him for a period of from several hours to one day before she comes into heat to the point

that she will allow service. In other animals, notably dogs, the pro-estrial period covers five to nine days.

Estrum, or the true heat period in cattle, is short in duration, covering a period of eighteen to twenty-four hours. At this time the animal manifests evidence of sexual desire and, in the absence of the male, will mount other cows in the pasture or corral. If confined by herself, she becomes restless and frequently bawls. There is a congestion of the vulva and at times a discharge of clear mucus from the vagina.

The metestrum is the period after the animal has passed out of heat and until the genital organs resume their normal state. This period offers no definite exterior manifestations, is of short duration in the cow, and is followed by the resting period, or diestrum, which covers much the greater portion of the estrual cycle, being eighteen to twenty-one days in length.

CHANGES TAKING PLACE IN THE GENITAL TRACT DURING THE ESTROUS CYCLE PHASES

Estrous cycle manifestations are brought on by the physiological activity of the genital organs, particularly the ovaries. The ovary is divided into two main parts, one termed the cortex or outer third, and the other the medulla or central portion.

In the outer third are situated the immature Graafian follicles in great numbers. Each Graafian follicle contains one ovum or female generative cell.

As each heat period approaches, one of these Graafian follicles enlarges and becomes mature. It then ruptures through the cortex of the ovary and its contained ova cell is discharged into the Fallopian tube. In the cow usually only one Graafian follicle ruptures at each estrum. In case two rupture and the animal is bred, twins will usually result from the service. In hogs, dogs, and other animals having a litter of offspring, a number of Graafian follicles become mature and rupture at each estrous period. After the follicle ruptures, the space it occupied in the ovary is filled with some red-blood cells, a connective tissue framework containing blood vessels, and large yellow-pigmented lutein cells. The term yellow body or corpus luteum has been applied to this structure. It is very large and may be readily seen on examining ovaries, e.g., at a packing house, as a bulging, yellow protuberance on the ovary.

The discharged ovum passes down the oviduct or Fallopian tube, which is a very small tube, about the size of a piece of baling wire, connecting the ovary with the horn of the uterus. At the time the follicle is maturing, the uterus is undergoing changes and its mucous membrane becomes congested preparatory to receiving the ovum and nourishing it in case breeding occurs and conception follows. If breeding does not occur, the ovum is discharged or becomes absorbed,

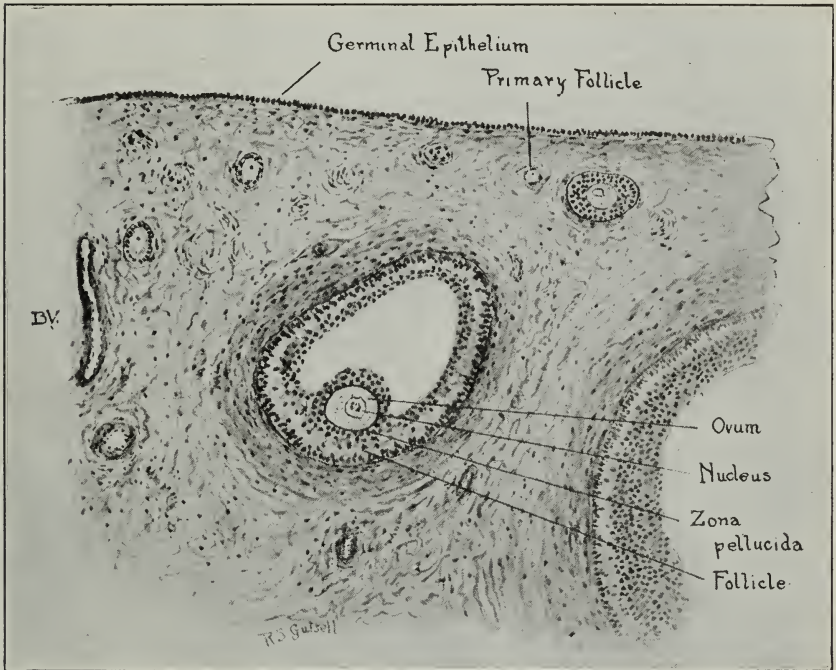


Fig. 9.—Microscopic section of ovary of cow showing a Graafian follicle with ovum. (After W. L. Williams.)

the congestion of the uterus recedes, and the animal passes into the diestrous period. Toward the end of the diestrous period, the yellow body undergoes absorption or possibly in some cases is expelled from the surface of the ovary coincident with the development of another Graafian follicle, and a new cycle is started. In case conception occurs at any heat period, the corpus luteum is not absorbed but remains permanently through the period of gestation. It is then known as the corpus luteum of pregnancy and no more heat periods appear under normal conditions until after the birth of the offspring. In the absence of conception, heat periods normally recur

with uniform regularity throughout the year at intervals of about twenty-one days, being, according to some investigators, slightly shorter during warm summer weather than in winter.

IRREGULARITIES IN THE ESTROUS CYCLE

While under ordinary conditions the presence of the estrous manifestations are a guide to the breeding status of the individual animal, too much dependence cannot be placed on this fact alone. The ideal breeding efficiency of cattle is obtained when each female gives birth to offspring every twelve months. Very few herds reach this standard and loss in efficiency of production in all herds, whether beef or dairy, increases as the breeding efficiency declines. This is one of the principal factors in the financial loss sustained from diseases of the genital tract.

Certain conditions cause complete and more or less permanent arrest of the estrual cycle in the absence of pregnancy. At times, although with much less frequency, estrum is manifested and service from the bull accepted one or more times during the period of an apparently normal gestation, followed by the birth of a healthy calf.

The condition of nutrition of an animal has an important bearing on the development of estrum. It is regularly observed, for example, in range cattle, during unfavorable years when feed is poor and weather conditions bad, causing mature, non-pregnant cows to get very thin, that no estrum is noticed until conditions are such that the animal begins to physically improve, even though months are required for this change to occur.

In experimental, small animals, notably the white rat, Evans¹⁹ has shown that by restricting the diet of the animals in certain ways the estrous cycle may be completely arrested or the animal may be kept more or less permanently in heat over a period of months. The normal estrous cycle in these animals covers a period of only ninety-six hours.

Hart, McCollum, Steenbock and Humphrey^{29, 30}, in Wisconsin, have been able to produce marked abnormalities in the breeding efficiency of cattle by restricted diets.

In sheep the lamb crop may be increased by the so-called "flushing" of the ewes at the breeding season.

In fact, we probably do not yet realize the full significance of diet on breeding efficiency, especially in dairy cows with high milk production records. Forbes²¹ and his assistants have definitely shown that the amount of inorganic salts, notably calcium and phosphorus,

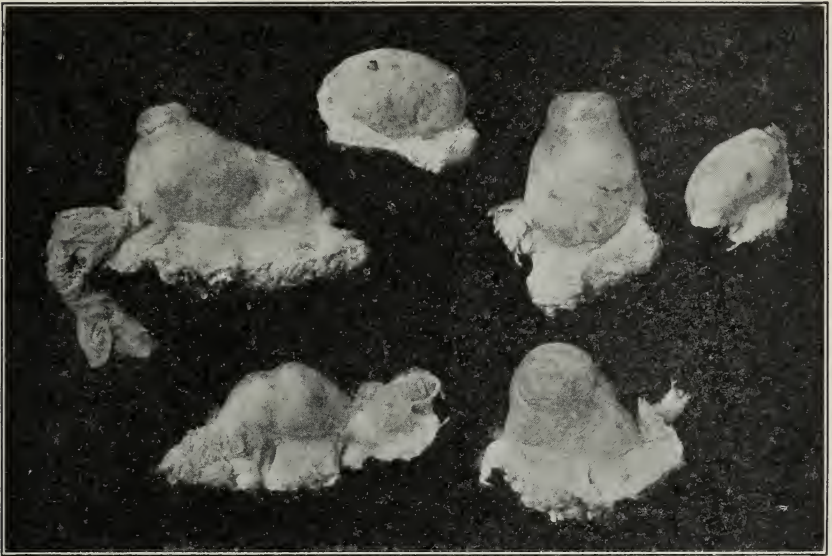


Fig. 10.—Three pairs of ovaries from cows, each pair showing an ovary containing a corpus luteum.

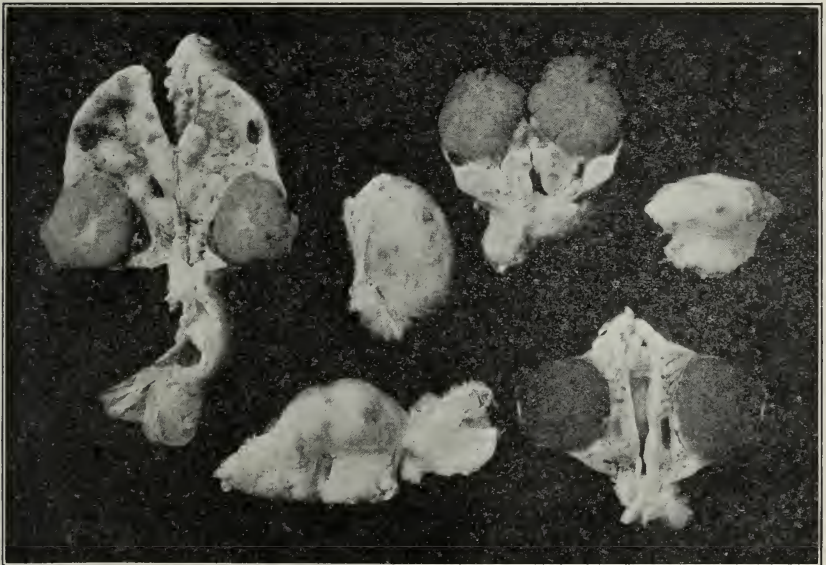


Fig. 11.—Same as figure 10, with the ovaries containing the corpora lutei cut in cross section.

given off by such animals in their milk in twenty-four hours and utilized by the body is more than they are taking in with their feed and they are therefore said to be in negative balance in regard to these elements. Studies by Meigs and Woodward⁴⁵ have shown the effect of shortage of these elements on the milk yield and how the latter may be increased by a long dry period and feeding of a diet rich in these substances. It is recognized that the phenomena of milk secretion are closely related to the activities of the genital organs. Just what effect such prolonged conditions of imperfect nutrition have upon the reproductive processes has yet to be fully ascertained by research and correlation of truths already elucidated.

One other condition which is definitely associated with the absence of heat periods is retention of the yellow body in the ovary. The cause of this condition is not recognized today and it may be intimately associated with imperfect nutrition. Normally, the temporary corpus luteum of estrum should become absorbed toward the end of the diestrus. If this fails to take place, development and maturation of another Graafian follicle does not occur, and no estrum appears. This condition occurs without any observable manifestations. The animal, following parturition, may come in heat regularly and be bred after two or three months. No further heat periods will be observed by the owner and he will judge the animal to be safely with calf, only to find after many months that the cow is low in milk production with no signs of advanced pregnancy. In other cases, after showing no signs of heat for a period of months, cows which are thought to be three to five months in calf, will again come in heat.

In extensive experimental work on the white rat, Long and Evans³⁹ found that providing this animal is bred at the estrous period and does not conceive, the next appearance of estrum will be delayed beyond the regular period for the cycle to recur. They showed that in this animal the act of copulation has an inhibitory effect on the development of the succeeding estrual cycle, and were able to produce the same effect by inserting a glass rod into the vagina at the estrual period, so that it touched the cervix. They were unable to produce this effect in guinea pigs, which animals have a longer estrual cycle than white rats. This may be an explanation for the observation made on a considerable percentage of cows in some herds that, following breeding at estrum without conception occurring, a period varying from thirty to fifty days will elapse before the animal again comes in heat.

No definite reason is at hand at present to explain the cause of so-called retained corpus luteum and it is not known whether this is the direct cause of retardation of the estrual cycle or whether some entirely different condition is the cause of which this is the only observable manifestation. However, when on examination per rectum of a cow supposed to be pregnant for several months the uterus is found to be empty, a corpus luteum of the last estrum will be found in one or the other ovary and can be readily felt when the ovary is palpated through the rectal walls. If the corpus luteum is expressed from the ovary, a new Graafian follicle will at once begin to mature in a very high percentage of cases and the animal will come into an apparently normal heat period in from one to six days, accept service and, in a good percentage of cases, become pregnant. When the corpus luteum can be easily expressed this may be done through the rectal walls. In some cases it is deeply embedded in the ovary and great pressure is required to remove it. In this case the other hand of the operator is placed in the vagina, the ovary is passed into it by the hand in the rectum and marked pressure made through the very strong vaginal walls without danger of injury to the animal. In a small percentage of cases a second, or even a third, attempt must be made to express a very adherent corpus. When it is removed, it drops free in the abdominal cavity of the animal and is absorbed.

It is these peculiarities of the estrual cycle under abnormal conditions which render a positive diagnosis of pregnancy in its early stages of such great economic value.

CHANGES IN THE GENITAL ORGANS OCCURRING IN PREGNANCY

When conception occurs following breeding at any estrual period, marked changes begin to occur in the genital organs, particularly in the uterus. The corpus luteum in the ovary discharging the ovum which became impregnated with the male cell remains through the period of gestation and no further observable activity takes place in these structures. On account of the fact that the uterus is furnishing a place for development as well as for nutrition of the growing offspring, the greatest changes are noted here.

At the time of copulation the semen containing the spermatozoa from the male is deposited in the vagina. The active motility of the spermatozoa, thousands of which are contained in the semen from a single service, causes them to pass up the cervical canal and through the uterus to the oviduct. Only one cell is necessary to impregnate a single ovum, and when one such male cell enters the ovum cell

conception has taken place and pregnancy becomes established. This is followed by a closing or sealing of the cervical canal, by means of the secretion by the lining cells of a firm translucent plug of tenacious mucus known as the uterine seal. This hermetically seals the interior of the uterus from the vagina.

The changes in the uterus consist in the multiplication and growth of the single celled ovum impregnated with a single male cell, from which the elaborate cellular structure and cell differentiation takes place to form the body of the offspring. During this development the fetus is covered by membranes known commonly as the placenta or afterbirth. Certain differentiated areas of the external membrane form the fetal cotyledons which attach to the maternal cotyledons on the inside of the wall of the uterus and in these cotyledons the interchange of waste products from the fetus and nutriment for its development takes place through the blood of the parent. The circulating blood of the mother and fetus do not mix in the cotyledons, but they are brought in such intimate contact with each other that interchange of waste products and nutriment takes place very much the same as occurs in the lungs of adult animals during the process of respiration. In the mare, there are no cotyledons as the placenta is diffuse.

During the fetal development the uterus must undergo gradual enlargement and its blood supply gradually increases. The bovine fetus lies in one or the other horn of the uterus, and this horn will gradually exceed in size the non-pregnant horn. In the case of twins, one may be located in each horn.

The uterus is supplied by three arteries, the utero-ovarian, the middle uterine, and the posterior uterine or vaginal. The middle uterine is the largest of these and is readily felt through the rectum in the folds of the broad ligament in non-pregnant animals and at all stages of pregnancy. The utero-ovarian artery lies anterior to this one and sends branches to the anterior part of the horn and the ovary. As the uterus enlarges during pregnancy this artery passes too far forward to be readily felt. The posterior uterine or vaginal artery can be felt at all stages of pregnancy but is also smaller than the middle uterine. The latter is therefore the artery which undergoes the greatest changes, is most readily felt, and is the one usually palpated for the information which the arteries give in regard to pregnancy.

After the fetus becomes formed, it lies in the amniotic fluid surrounded by the fetal membranes and is therefore easily movable as a floating mass in the liquid. It can sometimes be felt through the rectum as a firm movable mass when only six or eight inches long and in practically all cases at the end of the fifth month. Later, outlines of various parts of the fetus can be recognized, such as the head or extremities.

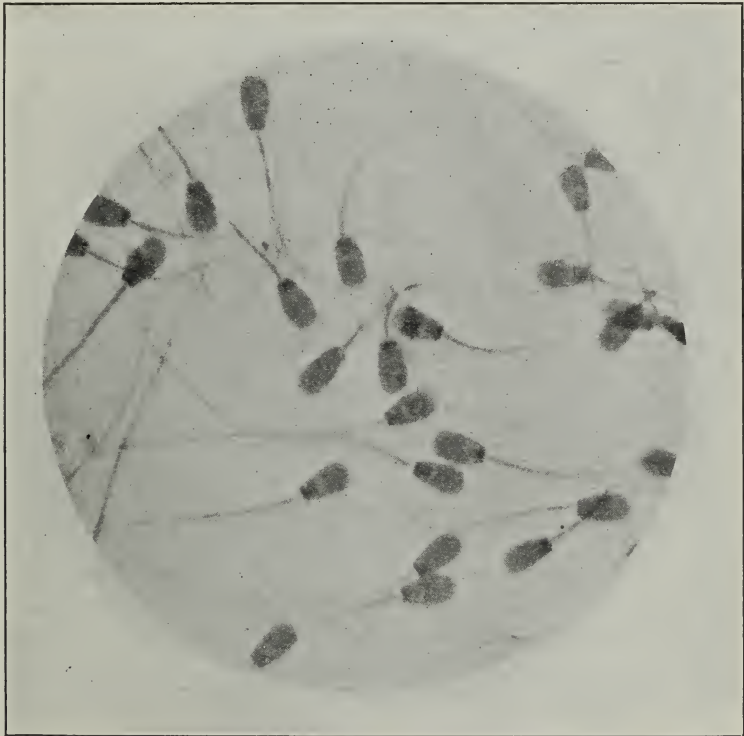


Fig. 12.—Microphotograph of spermatozoa from a bull.
Magnified $\times 800$.

At the beginning of pregnancy, the uterus and ovaries are entirely within the pelvic cavity. As gestation advances the uterus extends anteriorly and, at about the middle of pregnancy, falls forward over the brim of the pelvis. This carries the ovaries with it so that they cannot be felt per rectum. The anterior part of the pregnant horn late in pregnancy extends forward and downward, almost to the floor of the abdomen in some cases in old cows. At this time ballottement of the fetus can be obtained by abdominal palpation. There is a

fairly constant change in the character of the vaginal mucus during pregnancy. It tends to become more tenacious and translucent as compared to the stringy clear mucus of estrum. Microscopic examination of smear preparations of vaginal contents has been used with success by Long and Evans³⁹ in experimental studies on white rats to obtain information as to the stage of the estrous cycle.

We have carried this out daily over a period of months on ten cows, beginning a few days following parturition and continuing through the open period and for some weeks after the animals were again pregnant, without coming to any definite conclusion as to its value in the bovine. In such smears are found epithelial cells which line the mucous membrane of the genital tract, polymorphonuclear leucocytes, red blood cells, lymphoid cells, and mucus. Marked changes in the cell content were noted from day to day, but no definite changes denoting the approach or cessation of estrum, estrum itself, or the establishment of pregnancy, could be definitely detected. Considerable improvement in the technique of obtaining smears will be necessary before this can be definitely stated to be of no value for bovines.

The important changes to be noted in making an early diagnosis of pregnancy are a change in the character of the vaginal mucus and the formation of the uterine seal, enlargement of the uterus and horns, with the pregnant horn becoming larger than the non-pregnant, increased pulsation of the uterine artery, very frequently with a peculiar type of pulsation not found in other arteries. By careful observation of these factors an experienced man can diagnose pregnancy in from six to ten weeks after conception has taken place. This examination should be conducted with care. When the desired information can be obtained by rectal examination this is all that should be done. In bimanual examination, by which one hand is passed into the rectum and the other into the vagina, considerable straining may be brought on in the animal. The hand in the vagina, by grasping the cervix, fixes the uterus and with slight traction posteriorly will assist the hand in the rectum in outlining the body and horns of the uterus in some cases. There is some evidence that such examination at certain stages of pregnancy in some cows may be followed by abortion; this fact has already been noted in part III.

EXTERNAL MANIFESTATIONS OF PREGNANCY

The only external manifestation of pregnancy in the early months consists in an absence of heat periods, and for reasons already mentioned, too much dependence cannot be placed on this phenomenon. When external evidence must be depended on, therefore, it is generally considered that the animal must be six months pregnant or longer before definite conclusions can be drawn. The most valuable evidence is the feeling of the fetus through the abdominal walls by what is termed ballottement. This is done on the right side of the cow. The operator presses the thumb and forefinger of his right hand into the side of the flank with a sharp thrust and suddenly withdraws but still holds the hand against the animal's side, repeating the operation with momentary pauses between each thrust. This movement causes the fetus to move back and forth in the amniotic fluid in which it is floating and will cause it to strike the abdominal wall and give to the hand the sensation of a solid object in the abdominal cavity. This is done preferably when the animal is not engorged with feed or water—and movement of the fetus is more readily obtained in thin cows with comparatively thin-walled abdomens than in very fat animals. It is good evidence of pregnancy, although rarely an intestinal calculus or abdominal tumor may cause confusion.

Other evidence is the enlarged and pendulous nature of the abdomen, and a feeling of the movements of the fetus *in utero* through the abdominal wall, most commonly observed by the milker or other person in close association with the cow at frequent intervals.

ECONOMIC VALUE OF THE KNOWLEDGE OF THE PRESENCE AND STAGE OF PREGNANCY

In ordinary dairy operations a knowledge of these conditions is essential to economic production. Following abortion or breeding difficulties from a sterile or partially sterile bull plus the not inconsiderable amount of retained corpus luteum and delayed or absent estrual periods, the owner of a herd of dairy cattle may suffer a serious financial loss by not keeping informed regarding this point. When animals, particularly purebreds, are about to be sold at private sale or public auction and shipped long distances under a guaranty as breeders or as pregnant cows, the confirmation of this fact by an examination often relieves the seller from further responsibility or the buyer from accepting and shipping animals on the breeding history alone.

While in the past its application has been largely confined to dairy cattle, there are certain conditions under which it becomes of particular value in beef cattle. In culling out cows from stock cattle herds for slaughter, it is sometimes the practice to pick cows with no calves at their sides as barren animals and ship them, keeping the cows with calves because they are therefore supposedly breeders. This is by no means a sure criterion; the cow shipped may already be pregnant while the cow with calf at side may never again breed. Where bulls are constantly running on the range an examination of cows about to be shipped may reveal the presence of fairly advanced pregnancy, which would under certain conditions make it more economical to keep such animals. A decision on this point in addition to the condition of the animal might depend somewhat on market conditions and whether the market was rising or falling. Nevertheless, a man intending to remain in the cattle business would preferably dispose of cows that are not pregnant so far as this is practical. Especially would this be the case if the herd were being graded up by the use of purebred bulls where the offspring would be desired even if short feed conditions necessitated disposal of an unfinished steer in place of the pregnant cow.

Cattlemen having mountain ranges bring the cattle out in the fall. Cows not having calves running with them could then be separated. Lack of a calf might be due to abortion, the calf dying of disease, or being killed by predatory animals, or from the animal not having become pregnant. Many such cows become pregnant during the summer if bulls are on the ranges. If the existence of pregnancy and its duration are known, the owner can act intelligently in deciding whether to sell or keep the animal. One cannot definitely recognize the presence of pregnancy from simply looking at an animal until about the end of the sixth month or later, and yet a cow three to five months pregnant frequently might be kept if this fact were definitely known. When cows go into winter quarters and calves are separated, the cows could be examined for pregnancy. Those which are not pregnant could be turned into good pasture or fed in feed lots to make spring beef, while cows which are pregnant and intended to be returned to the range the following summer need not be fed so well when feed is scarce and high.

By working out a system of this kind it would seem that the percentage calf crop of range herds could be increased with profitable results. The examination of range cattle is very practical when a chute is at hand for the purpose and they stand quietly for the operator after he gets in the chute behind them. Climbing in and out

of the chute for each examination is tiresome but one man can examine from fifty to one hundred head of cattle per day in this way. When a considerable number of cows are being examined for pregnancy in this manner, with rectal examination only a certain percentage of error will occur from various causes, but this will not be sufficient to reduce seriously the value of the work.

Rounding up and handling animals for this examination, particularly when they are widely scattered on the range or when feed conditions are very poor, may be too hard on them to justify the procedure, and such factors should always be taken into consideration by stockmen in weighing its advantages and disadvantages.

SUMMARY AND CONCLUSIONS

The estrous cycle in cattle covers a period of about twenty-one days and, under normal conditions, recurs rather regularly.

Absence of estrual periods even following service cannot be taken as positive evidence of pregnancy.

A small percentage of cows may come into estrum and accept service one or more times during an apparently normal pregnancy.

A knowledge of the existence of pregnancy from about the end of the second to the end of the sixth month can only be definitely determined by rectal or vaginal examination, or both. From the end of the sixth month pregnancy can usually be determined from abdominal palpation and other external physical signs.

At attempt has been made by us to diagnose pregnancy prior to the end of the second month by microscopic examination of the cells in vaginal smears, but so far without success.

A knowledge of the presence and duration of pregnancy is of great economic value in both dairy and beef cattle under certain conditions.

VI

SCOURS AND PNEUMONIA IN CALVES

BY GEORGE H. HART AND JACOB TRAUM

INTRODUCTION

Practically every veterinarian and livestock man interested in dairying and purebred cattle has had experience with the diseases of calves which occur in the early days of their lives. A great variety of ailments has been reported. The most important disease is commonly known under the terms of white scours, calf scours, calf dysentery, or calf diarrhoea. Most writers on calf diseases describe calf pneumonia and navel infection under separate headings. While these three diseases may appear independent of each other, they are at times found closely associated. This is particularly the case with white scours and pneumonia.

White scours has further been divided, according to the microorganism that is found, into various groups. Jensen³⁶ established groups according to the organisms found, but also made two large divisions based upon the presence or absence of the bacteria in the blood and other internal organs. In 251 cases examined in his laboratory the following bacteriological findings were made:

Bacterium coli with bacteremia	118 cases
Bacterium coli without bacteremia	53 cases
Bacterium paracoli	16 cases
Bacterium coli and paracoli	3 cases
Micrococci	18 cases
Bacterium proteus	11 cases
Bacterium abortum	1 case
Unclassified infections	31 cases

There seems to be no doubt that various organisms can set up disturbances under different conditions and the disturbances may differ from each other. The difference, however, is not always well marked and the microbial cause not definitely settled. Therefore it is well to discuss scours as a whole, irrespective of the predominating organisms. Williams⁹⁰ goes still further and treats the whole subject of calf diseases under one heading, including calf scours, pneumonia, arthritis, and navel infections. All observers agree that

these may coëxist or merge into each other in such a manner that it is very difficult to establish a line of demarcation. We consider calf scours to be the most important cause of loss in young animals and will therefore confine the present discussion largely to this disease, while also taking recognition of the importance of calf pneumonia.

BACTERIAL CAUSES

It is very evident that bacteria have been given a prominent rôle in the production of the disease; in fact, there is little doubt that micro-organisms are principally responsible for the disease and the damage wrought, no matter what may be the predisposing causes. The latter will be discussed separately. It is further noted, from what has been previously stated, that more than one kind of micro-organism has been held responsible for calf scours, and while one kind of organism may be found as the causative agent on a given dairy or breeding establishment, no investigator holds that one organism alone is the all-important microbial factor in scours of calves.

The first bacteriological studies on this disease were reported in 1891 by C. O. Jensen,³⁶ of Copenhagen, and to date this investigator and his colleagues have been most prominent in the study of calf diseases. Jensen incriminated at first *Bacterium coli*,* which he found regularly in the blood and internal organs of calves that had died from scours. He claims to have satisfactorily infected healthy calves by feeding them small amounts of cultures of *Bacterium coli* isolated from cases of scours, and to have produced the typical scours' picture. He could not, however, produce disease with *Bacterium coli* isolated from the intestinal tract of normal calves.

The Italian investigators, Piana,¹⁵ and Monti and Varatti,¹⁵ agreed with Jensen, except that Piana's inoculation experiments were not successful in producing the disease. Mazzaniti and Viggezi¹⁵ reported finding a diplococcus in the cases of scours examined by them.

Poels,¹⁵ of Rotterdam, published an extensive report of diseases of calves in 1899. In this he agreed with Jensen's findings as to the causative agent, but also called attention to the fact that scours may be due to other organisms, especially to a *pseudocolon*.†

* Jensen's table of carbohydrate reactions shows that he encountered both *Bacterium coli communis* and *Bacterium coli communior*.

† *Pseudocolon* was classed by Jensen with his *paracoli*, but Titze and Weichel found this organism to agree with *Bacterium coli* in all respects except that it did not coagulate milk nor produce indol in peptone bouillon.

The French investigators Lesage and Delmar¹⁵ were in entire disagreement with the views of the above-mentioned men. They recovered from diseased calves an ovoid bacterium belonging to the Pasteurella group. They were unable to produce the disease by giving their organism in food, but could, by intravenous inoculation, cause a diseased condition resembling field cases of scours but not altogether like them. Lesage and Delmar claimed that the colon organisms were present in the intestines and reached the blood and other organs in the agonal stages of the disease and were not responsible for the disease.

Nocard^{54, 55} investigated a disease in Ireland for which he coined the name "white scours." His description of the disease differed somewhat from the findings of the authors previously mentioned. According to Jensen, Nocard was dealing with a navel infection. At the beginning of the investigation he did not come to any conclusion as to the bacterial cause since he found *Bacterium coli*, *pseudocoli* and other micro-organisms in the tissues examined. Later, however, from a calf showing arthritis, he isolated an organism belonging to the Pasteurella group similar to the one isolated by Lesage and Delmar.

Titze and Weichel⁸¹ laid a good deal of stress on *Bacterium paracoli*,* isolated by them, as a prominent factor in the scours of calves. Before the report of their work, this bacterium had already been found by Jensen and others. Titze and Weichel proved rather satisfactorily the power of *Bacterium paracoli* to produce typical cases of clinical scours in an experiment upon four calves. Two of these calves, each two days old, were fed cultures of this organism. With each in a separate stall was placed an eight-day-old calf as a control. The infected calves developed typical scours and later the control animals also developed the scours by association. In another feeding experiment upon two calves, twenty and thirty-one days old, respectively, they were again successful in producing the disease with cultures of *Bacterium paracoli*.

Krautstrunk,³⁸ in 1909, in seventy-three calves examined found nine cases in which diplococci were obtained in pure culture from the blood and other organs. In sixteen cases diplococci were associated with *Bacterium coli*. In a series of investigations in which four animals were fed cultures of diplococci, two injected into the umbilicus and two others injected intravenously, he proved that this

* This organism agrees with Gaertner's *enteritidis* organism and with *Paratyphoid B.*, but differs from the former in serologic tests and from the latter in both serum reaction and in its pathogenicity for human beings and cattle.

organism is capable of producing the disease. These eight calves were infected on the day they were born, they had not partaken of any food and were placed in a stall in which calves had never been kept before. He was able to infect two newly-born calves with cultures that were nine months old.

Christensen,¹⁶ in a summary on paracolibacillosis of calves, found that 37.8 per cent of calves so affected were thirteen days to one month old, 27 per cent were one month and over, and only 7 per cent were under eight days of age.

Meyer, Traum, and Roadhouse⁴⁸ reported an outbreak of infectious diarrhoea of calves caused by *Bacterium paracoli*. The calves were being used in a feeding experiment. The ages of the calves that sickened ranged from twenty-four to eighty-seven days.

In view of the fact that varieties of *Bacterium coli* and of most of the other organisms associated with calf scours have a wide natural distribution and have frequently been found in the intestinal tract of normal calves, most investigators, including Jensen and Titze and Weichel, made search for filterable virus in order to reassure themselves that the organisms found by them were not playing the rôle of secondary invaders. The necessity was especially impressed upon them when the filterable virus was accepted as the cause of hog cholera instead of the hog cholera bacillus, which is very much like the paracolonic organisms mentioned above. By experiments on calves, the above-mentioned workers failed to demonstrate the presence of a filterable virus.

Until recently the diseases of calves have received very little study in this country. The findings of the investigations reviewed here have been accepted as having sufficiently solved the question. Control measures based upon these findings were adopted and found satisfactory to a large extent, but such measures failed in an important percentage of outbreaks of calf diseases. This failure stimulated renewed investigations concerned not only with the variety of bacteria found but also with the source of infection. We will therefore discuss the results of the recent work under "Avenues of Infection."

Without going into detailed experimental evidence on the bacteriology of pneumonia in calves, an idea of the microorganisms found in this malady may be obtained from the following list of ninety-eight autopsies and bacteriological examinations on calves affected with the disease made by Jensen³⁷ and his co-workers.

- 6 cases of septic pleurapneumonia with *Bacterium vitulasepticus*.
- 2 cases of pneumonia with fine ovoid bacillus.*
- 46 cases of pneumonia with *Bacterium paracoli*.
- 14 cases of pneumonia with *Bacillus pyogenes*.
- 20 cases of pneumonia with mixed bacterial flora.
- 4 cases of pneumonia without demonstration of bacteria.
- 4 cases of embolic pneumonia.
- 2 cases of strongylosis pneumonia.

In this country Hagan²⁶ found *Bacterium coli* associated with pneumonia. Carpenter and Gilman¹³ found *Bacterium bovissepticus*, *pseudomonas pyocyaneus*, streptococci and organisms of the colon aerogenes group in the cases they studied. In twenty-nine cases studied by Theobald Smith,⁷⁶ *Bacterium actinoides* was incriminated.

AVENUES OF INFECTION

In formulating recommendations for the control of any infectious disease, it is not only necessary to know the kind of organism that is concerned, but it is equally or even more important to know how it reaches its victim. Practically all the investigators above-mentioned discuss this phase of the question. Jensen held that the most important avenue of infection is the digestive tract and that the infection occurs after the calf has been dropped. Poels agreed with him, but maintained that the infection probably is picked up as the calf passes through the vagina and that infection by way of the ruptured umbilical vessels also takes place.

Lesage and Delmar and later Nocard could not produce disease by feeding the Pasteurella which they incriminated as the causative organism. They could, however, set up a process of disease by intravenous inoculation, so they concluded that the infection must take place through the ruptured umbilicus. Lesage and Delmar explained infections in older calves as taking place through the respiratory system.

Titze and Wiechel have proved that infection does take place by way of the digestive tract.

In the particular outbreak reported by Meyer, Traum, and Roadhouse, infection by the digestive tract appears to be the only plausible explanation, since infection spread to various pens of animals which were old enough for the umbilical stump to have dried, dropped off.

* These organisms were very much like *Bacterium vitulasepticus* except that they were not pathogenic for rabbits and mice.

and the wound to have healed. Infection could hardly have been charged to preëxisting infection since the animals came from different ranches.

Up to the time of the work of Williams and his colleagues, it was generally accepted that infection entered either through the digestive tract or the umbilicus. Those believing the former to be the important path of infection admitted that infection by the umbilicus was possible, but claimed that it gave rise to the entirely different disease known as navel or joint ill.

Nocard,⁵³ from his studies on infectious abortion in cattle, concluded that diseases of new-born calves were intimately associated with abortion, and claimed that infection of the calves takes place in the uterus. Franck, before him, also held this view. Nocard, however, in his report of investigations on calf scours in Ireland maintained that the animals became infected through the umbilicus, and it was not until Williams emphasized the possibility that fundamentally the diseases of new-born calves are intra-uterine infections, that this mode of infection was given serious consideration.

Williams and his co-workers made the observation that, as soon as the pharynx is properly formed, the fetus swallows the amniotic fluid, which is promptly absorbed by the intestines, and the solids, such as hair, bacteria and epithelial scales, remain in the meconium. They further demonstrated that there may be fetal diarrhoea by finding pellets of meconium in the gastro-intestinal tract of aborted fetuses, and that the latter were not rarely covered with fecal matter. They deemed it very desirable to know the nature of the bacterial flora of the meconium, and Hagan²⁶ did some work to determine this. His results are rather interesting, though not conclusive. He worked with meconium samples from two herds. In one herd, where they had very little calf diarrhoea, six calves were available. The meconium was sterile in all six specimens. In the other herd, he studied eight samples. In this herd, there had been considerable calf scours. Three specimens were sterile, three contained *Bacterium coli*, one showed streptococci, and one streptococci and staphylococci.

The source of these bacteria in the sealed uterus was the next problem that confronted Hagan. He examined material received from packing houses and endeavored to determine the bacterial flora of the utero-chorionic space, of the fetal fluids, and of the fetal blood. The surprising conclusion was that 87 per cent of the samples taken from the utero-chorionic space yielded cultures and about one-half of the bacteria recovered were of the colon group. The next largest

variety of organisms were streptococci. He found that in the fetal fluids only about 30 per cent of the samples contained organisms, and concluded that there was a barrier somewhere preventing the bacteria from breaking through into the fetal fluids. The fetal blood specimens were practically all sterile.

A similar work was reported by Giltner and Bandeen,²³ but with different results. They made a bacteriological study of ten uteri and the meconium of calves which were contained in these uteri. They were working with cases of metritis, and it is only natural to expect that the bacterial flora in their cases would be entirely different from those examined by Hagan. Giltner and Bandeen were unable to reconcile the bacterial findings in the uterus with the findings in the meconium.

C. M. Carpenter¹² conducted a series of bacteriological examinations from which he had hoped to determine (1) the organisms that normally live in the female genital tract of cattle; (2) the age at which such organisms enter the genital tract; (3) the relation that exists between the organisms and the pathological conditions associated with them; (4) the effect that such organisms produce on the fetus *in utero* or on the calf after birth. His results failed to permit any definite conclusions; in fact, he had difficulty in interpreting his findings on the theory of intra-uterine infections. He states: "It seems as if there must be some unknown factor at work in producing these morbid conditions in the reproductive organs of cattle and in the life of the new-born. I cannot see very much etiological relation between these conditions and the majority of organisms isolated." Carpenter's findings are at considerable variance with those of Hagan. In ten normal pregnant uteri, the former obtained no growth in six, or 60 per cent, whereas Hagan found only 13 per cent of twenty pregnant uteri showing no growth. Further, Carpenter did not find *Bacterium coli* in any of the uteri examined by him.

In summing up the great amount of research done on this disease, the conclusion to be reached is the fact that certainly more than one variety of organism is associated with outbreaks of the disease and that infection usually takes place through the digestive tract after the birth of the calf, and less frequently through the umbilical vessels and *in utero*. In individual outbreaks one avenue of infection may clearly stand out as the predominating route in those particular cases.

Another outstanding fact is that while white scours is frequently associated with infectious abortion, and in the minds of many live-stock owners and veterinarians has a relationship to this disease,

Bacterium abortum is not found associated with it in a way to incriminate this organism in a causative relationship. This is shown by the fact that Jensen found it in only one of 251 cases examined. We have been unable to find any published reports of *Bacterium abortum* producing the disease experimentally. The presence of *Bacterium abortum* infection in the mother, however, is responsible for the birth of premature and weakened calves which are more susceptible to the factors which bring on this disease. In abortion-infected herds retained afterbirth, pyometra, and other conditions cause the genital tract to become infected with other organisms. These pass out with the vaginal discharges and may run down the thighs and udder of the cow to the teats from which they can readily be taken into the gastro-intestinal tract of the calf. They may reach the mouth and stomach of the calf from its licking or picking up litter from badly contaminated surroundings. Practically all the varieties of bacteria found in affected calves are more or less widely distributed in nature, and when this disease is present other factors are probably essential in causing them to assume the pathogenic rôle leading to the development of the disease. It is evident from our present knowledge that it is not a specific infection, and means cannot always be developed to eliminate the organisms which are usually found associated with it. This makes all the more important a knowledge of predisposing factors and means of eliminating these.

INCIDENCE OF THE DISEASE

This disease is largely prevalent in dairy herds, and the offspring of purebred and highly developed grade herds seem to be more susceptible than those from ordinary cattle. In purebred beef herds it is found especially when the animals are closely housed. Under range conditions and in ordinary stock cattle herds, despite occasional outbreaks it is rarely a factor of importance. It is comparatively rare in small herds and the incidence of the disease, severity of the cases, duration of its prevalence, and difficulties of controlling it seem to increase with the size of the herd. Seasons of the year appear to have little effect on its development. In large herds where it is more or less endemic, extremes of weather conditions seem to increase its prevalence and enhance its virulence. Thus it becomes more serious in hot summer weather, severe rainy weather, and cold winter weather. In herds containing hundreds of head of dairy cattle it has been observed to continue throughout an entire year or even a longer period. In such herds, calf pneumonia, arthritis, and all other forms of calf diseases

are frequently associated with it. Under such conditions 75 to 90 per cent of the calves may be attacked and from 50 to 100 per cent of the cases have a fatal termination. The losses may be so severe that it may become impossible to maintain the numerical strength of the herd save by the purchase of outside cattle.

FACTORS ASIDE FROM BACTERIAL AGENTS IN THE CAUSATION OF THE DISEASE

These conditions, of which there are a number, may properly be classed as predisposing factors and are of very great importance. Mention has already been made of the presence of abortion infection as a factor. In abortion-infected herds, calves born slightly premature and at term but in a weakened condition are more prone to develop white scours than would be the case did *Bactèrium abortum* infection not exist in the genital tract of the dams.

Recently published work by Smith and Little⁷⁸ rather definitely shows that feeding of colostrum from the dam is essential to the prevention of losses under some conditions. This idea was originally advanced by Jensen in 1905, who found that calves died of enteritis when fed heated milk during the first twenty-four hours, which could be obviated by supplying colostrum from the dam for one day or longer. In the work of Smith and Little, ten calves fed colostrum all survived the danger period, although three died at twenty-five, thirty-eight, and forty-five days, respectively, probably from some kind of poison. In a second group of twelve calves receiving no colostrum, nine died and three survived. Seven of the nine died within six days. It would therefore seem from this work that, under certain conditions, e.g., where the prevention of tuberculosis infection is desired and it thus becomes necessary to at once remove the calf and feed it pasteurized milk, some forms of scours may be prevented by giving normal lactating cow serum to the calves by injection or feeding.

The remainder of the predisposing factors may all be grouped under the heading of improper calf husbandry, and too much stress cannot be laid on their importance. To eliminate them includes proper sanitary measures at the time of birth, followed by proper feeding and housing of the new-born calf during the first weeks of its life. The degree to which preventive measures must be carried depends on the amount and severity of infection. Marked results in stopping the development of new cases of this disease have been

obtained by Duebler¹⁷ in Pennsylvania through the construction of a maternity hospital consisting of from one to several stalls, depending on the size of the herd. The building is so constructed that each stall opens to the outside only and can be thoroughly disinfected or fumigated following each maternity case that is placed in it. This is not a laborious practice to carry out when the building is available, and it prevents the new-born from coming at once into contact with infected surroundings, as is so generally the case in ordinary dairy farms where the disease has been prevalent for weeks and months. Complete success would not be expected to result from it in those cases where infection took place in intra-uterine life or at the time of birth. Such infections, as already mentioned, are probably greatly in the minority. Accompanying such precaution, the navel should be treated with tincture of iodine as soon as practicable after birth and kept dusted with drying powders.

Feeding of the calf is important. It is generally believed that it is essential for the calf to get the colostrum from the mother in order for it to expel the meconium. In raising healthy calves from tuberculous cows, this is not desirable on account of the danger of transmitting infection. We have seen new-born calves fed only pasteurized milk without any evidence of constipation or other harmful results. There was, however, no evidence of scours existing on this farm at the time. In outbreaks of the disease, better results are obtained in some cases, when it is practical, by letting the calf nurse the mother during the first ten days or by feeding it raw milk. This statement is based on the experimentally-proved fact that the milk of the mother in the first days of lactation, especially the colostrum, contains protective antibodies for infections that might be transmitted from the mother to the offspring.

It is important that new-born calves be not given too much milk during the early period of their lives. Our dairy cows are developed to milk production far in excess of that needed to sustain the life of the offspring. For this reason there is considerable danger when young calves run with, and especially, are turned in with their dams twice daily, that they will get too much milk. When calves are being pail-fed, over-feeding seems to be even more serious. When the disease is prevalent in a herd, the amount of pail-fed milk must be kept down to a minimum, even as little as two pounds of whole milk twice daily for the first several days being recommended. It is essential that the milk at the time of feeding be as near body temperature as possible. It must be fresh and free from excessive numbers

of bacteria. The latter condition can only be assured by handling the milk in properly cleaned and sterilized dairy utensils, and the calf pails should be looked after in this regard as scrupulously as any of the dairy tinware.

Housing of the young calves should have for its object their protection from the direct heat of the sun in summer and from rain, drafts, and winds in winter. At the same time the quarters must be so constructed that they can be readily cleaned and the calves handily fed. It is preferable to so arrange the interior that there can be individual pens for calves with sides to these pens which can be removed at will.

The losses from this disease are sometimes the most discouraging factor with which breeders are confronted. A little extra capital invested in equipment to properly handle calves is money wisely spent. Even when this has been installed, it is the all too common practice on a high percentage of ranches to select a calf attendant without any consideration of his ability for the work. The handling of calves during the early weeks of their lives is a matter requiring patience and scrupulous attention to details, in addition to experience. This can only be found in a better type of man than is usually employed for the purpose on a majority of our large dairy ranches. Owners will stop losses from this disease when they more fully realize that the position of calf attendant is one of the more important on the dairy. Good calf attendants are all too scarce today and the filling of the place requires the personal attention of the owner. Frequently serious losses among healthy calves, or great reduction in the incidence of the disease on infected premises, have been observed to follow a change in calf attendants.

PREVENTION

Calf scours is a disease of small consequence to the breeder having small groups of cattle. Such men may remain in the business for years and never experience difficulty in raising calves. Few or no preventive measures are needed in such cases. It is often men of many years' experience of this kind that are inclined to belittle the precautionary measures necessary to success on large breeding establishments. With the increase in size of the herd, the danger of the disease making its appearance increases, and no dairy herds numbering hundreds of breeding animals will escape its ravages unless cognizance is taken of the factors aside from bacterial agents in the causation of the disease which have been outlined in the preceding pages.

Medicinally, one biologic product is on the market for the prevention of this disease, known under the name of calf scours serum. This serum is obtained from horses immunized with strains of the various organisms isolated from outbreaks of the disease in calves. As a preventive measure, 10 to 20 mils of this product are given subcutaneously to the calves as soon as they are born and repeated one or more times. In some herds this has been very successful in prevention, while in others little or no benefit has been derived from its use. In some cases a special serum prepared from strains of organisms isolated from the affected calves on a particular ranch has been prepared and used with greater success than was obtained with the stock calf scours serum on the market.

Jensen, of Copenhagen, has developed this specific serum production to a high degree. On a great many farms in Denmark they have indexed the kind of organisms found on the individual farms, and have a specific serum for each farm. In case they have a call for serum from a farm for which they have not developed a specific strain, they give a polyvalent serum and endeavor to isolate the organism causing those particular cases. If serological tests show that they have a serum that will check the trouble, they use that serum; otherwise, another horse is immunized against the particular strain. The Jensen method has met with great success. The investigators over there have worked with this group of diseases so long that the procedure has become a regular routine, and they seem to be well satisfied with their results.

On those farms where calf pneumonia is prevalent, the use of calf pneumonia bacterins is recommended.

TREATMENT

The prevention of this disease is more important than treatment, and when large numbers of calves are being handled it frequently becomes impossible to devote a great amount of time to individual cases. The first step in treating this disease is to completely withdraw milk from the diet for a period of twenty-four hours. Warm water, water containing one or two raw eggs, or barley water, may be substituted. Milk may then be added to the diet but given very sparingly, and its effect observed.

Calf scours serum may be used as a curative agent in doses of 50 to 100 mils or more if the value of the calf warrants the cost.

Alkaline treatment, as recommended by Frost and Varley, has given good results in some cases in competent hands. This treatment

is based on human experience, that where the feces in cases of diarrhoea in the new-born child are not allowed to remain acid, fatalities are exceedingly rare. Normally, the feces of the calf should be alkaline or neutral, and when scours develop they soon become acid. Acidity is determined by testing the feces with ordinary litmus paper. When acidity is present, one ounce of sodium bicarbonate and two drams of aromatic spirits of ammonia in one pint of warm water should be given one hour before feeding, night and morning, as an antacid and stimulant.

In cases of calf pneumonia, good results have followed the use of calf pneumonia bacterins. Especially have such procedures resulted favorably when the organisms causing the infection on the particular farm are used in the bacterin. When pneumonia tends to develop in calves recovered from scours, these bacterins should be given as soon as the animals are safely out of danger from the diarrhoea.

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GLOSSARY

- Aerobically. In the presence of oxygen.
- Amnion. Innermost membrane covering the fetus.
- Amniotic fluid. Fluid contained in the amnion which surrounds the fetus.
- Atresia. Absence of or narrowing of a normal opening.
- Bacillus. Rod-shaped bacteria.
- Bacteremia. The presence of bacteria in the blood.
- Cervix. The neck of the uterus opening into the vagina.
- Chorionitis. Inflammation of the chorion or external membrane covering the fetus.
- Chorion. Outermost membrane covering the fetus.
- Cocci. Spherical-shaped bacteria.
- Coruna. Horn. Applied to the right and left horns of the uterus.
- Corpus luteum. The yellow body which fills the cavity in the ovary from which the ovum is discharged.
- Cotyledons. The points of attachment of the fetal membranes to the wall of the uterus.
- Desquamation. Separation or casting off of the surface of any membrane.
- Diphtheroid. Applied to bacteria resembling the diphtheria bacillus.
- Diplococci. Spherical-shaped bacteria growing in twos.
- Endometritis. Inflammation of the lining membrane of the uterus.
- Endometrium. Membrane lining interior surface of uterus.
- Epididymis. A part of the testicle.
- Fallopian tubes. Same as oviduct. Tube connecting ovary with horn of uterus.
- Fimbriated. Fringed-like end of oviduct surrounding ovary.
- Graafian follicle. Spherical body in ovary containing the ovum.
- Hemolyze. Dissolution of red blood cells.
- Medulla oblongata. Part of central nervous system connecting spinal cord and brain.
- Metritis. Inflammation of the uterus.
- Oviduct. Tube connecting ovary with horn of uterus.
- Pasteurella. A group of short polar staining bacteria.
- Per os.* By the mouth.
- Placenta. The membrane within the uterus which establishes communication between mother and offspring.
- Placentitis. Inflammation of the placenta or fetal membrane.
- Polyvalent. Capacity of resisting infection with more than one organism or strain of bacteria.
- Pseudomonas. A motile bacteria with a single hairlike process at one end.
- Salpingitis. Inflammation of the tube connecting ovary and horn of the uterus.
- Seminal vesicles. Pouches in the male genital tract for the collection of semen.
- Spermatozoa. The male reproduction cell.
- Spirilla. A coil-shaped bacteria.
- Streptococci. Spherical bacteria growing in chains.
- Vibrios. Similar to spirilla. A coil-shaped bacteria.

